International Journal for Multidisciplinary Research (IJFMR)

E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Development of Amino Acid-Rich Protein Supplement to Stimulate Protease for Muscle Growth

Akshatha[1], Vasumati Sri. P[2], Anit Thomas[3]

[1]Assistant professor, Department of Clinical Nutrition and Dietetics, Padmashree Institute of Management and Sciences, Bangalore

[2,3]Master of Science, Department of Clinical Nutrition and Dietetics, Padmashree Institute of Management and Sciences, Bangalore

Abstract

Legumes are excellent sources of protein, carbohydrates, and minerals, and their nutritional quality can be enhanced through processing methods like soaking and cooking. Combining legumes with cereals improves their amino acid profiles, making them increasingly popular as plant-based protein options in response to dietary trends and health concerns. Bromelain, a protease from pineapples, is extensively studied for therapeutic use and can be economically sourced from pineapple waste. The present study aims to develop a protein supplement with the incorporation of bromelain to meet the requirements of amino acid post-workout for a better outcome for the muscle. The methodology included a selection of ingredients along with flavouring ingredients. The samples were pretreated by soaking, germination, and dehulling while developing protein powder consistency and its fruit flavors were adjusted. A sensory evaluation reveals that pineapple fruit flavour seems to be the most acceptable. Nutritional analysis was determined using standardized analytical techniques showing the supplements had notable protein content (26.60 g) and low fat (0.38 g). A gelatinase test verified the presence of protease enzymes in the fruit powders, highlighting their protein-breaking capabilities. The developed protein supplement with pineapple offered a well-rounded nutritional profile, improved digestion with proteolytic enzymes and received high marks in sensory tests, presenting it as an attractive and health-conscious dietary choice.

Keywords: Protein supplement, Pineapple, Amino acids, Muscle build, Bromelain.

Introduction

Protein requirements in the diet are higher for athletes than for non-trainers. Research suggests that the protein requirements of these athletes are between one and two times higher than the recommended daily allowance. Complete proteins are defined as protein sources containing all the essential amino acids, while incomplete protein sources lack any of the essential amino acids (Chad *et al*., 2006). Plant-based proteins have been identified as having less adverse effects on the environment and the economy than animal proteins. Their two main disadvantages however are the absence of some important amino acids required to meet human nutritional demands and the inclusion of anti-nutritional elements which hinder digestion. Compared to animal-based protein, legumes have lower digestibility but contain a high amount of protein, carbohydrates and minerals. The lower digestibility of the proteins in legumes is due to their antinutritional

factors and high protein storage capacity. Various processing methods such as soaking, germination, cooking, and roasting improve the nutritional quality and digestibility of protein (Alberta *et al.,* 2016). Legumes and cereal proteins are typically combined to form a more nutritionally complete amino acid profile, which enhances the nutritional qualities of plant-based protein sources (Reynauda *et al.*,2020).
In the food industry, plant-based protein ingredients are becoming more and more popular because consumer trends in recent years have driven product development and reformulation away from the use of animal-derived proteins such as casein, whey, and egg. Over dietary preferences based on supply, cost, morality or health, cereals and legumes are generally rich sources of fiber, vitamins, minerals, proteins, and other phytonutrients (Andrea, *et al.*, 2019). Lentils have high nutritional content and cook quickly, there has been an increase in interest in their production and application in food formulation Proteins, vitamins, minerals, and carbohydrates (such as fiber, resistant starch, and oligosaccharides) can also be found in good amounts in lentils because of their high content of amino acids like arginine and lysine, lentils can enhance the nutritional value of cereal proteins by themselves (Barbana *et al*., 2013).
Out of all the enzymes, proteases play a vital role, enzymes have two important characteristics for therapeutic use one is great affinity & specification and the other is targetability to convert into desired products. Protein digestion starts in the stomach through proteases; they are used in therapeutic purposes from simple disorders to complex diseases derived from zymogen. (Shankar *et al.,* 2021). Among the various enzymes, bromelain refers to a group of proteolytic enzymes found in plants of the *Bromeliaceae* family, particularly prominent in the pineapple (Ananas comosus). While other proteolytic enzymes such as ananain and comosain exist in pineapple, bromelain is the most extensively studied. It is abundant in the stem and fruit of pineapples. Bromelain extracted from the fruit is known as fruit bromelain (FBM), while that from the stem is stem bromelain which has been commercially developed and studied for its potential medical applications. Researchers are also exploring the use of pineapple waste as an economical source of bromelain (Varilla *et al*.,2021)**.**
Hence, the present study aimed to develop a protein supplement along with the presence of bromelain to meet the requirements of amino acid post-workout for the better outcome of the muscle.

Material and Method

Materials

The selected ingredients field bean, rajmah, amaranth and black gram were purchased in the nearby local supermarket. Pineapple, Papaya and kiwi were bought from the local fruit shop in Bangalore, which has naturally present proteolytic enzymes in the fruits**.**

Materials and Method

Materials

The selected ingredients field bean, rajmah, amaranth and black gram were purchased in the nearby supermarket. Pineapple, Papaya and Kiwi were bought from the local fruit shop in Bangalore.

Methods

The selection of ingredients mainly focuses on its protein and amino acid profile as shown in Table no 1 based on the digestibility present in field beans (*Vicia faba L.)*, red kidney beans (*Phaseolus vulgaris L.*), black gram (Phaseolus mungo) and amaranth seeds (*Amaranthacae*). Similarly, the selection of the fruit was based on the presence of proteolytic enzymes such as bromelain, papain and actinidain. Pretreatment processes such as soaking and germination were carried out at different durations followed by dehulling for further extraction of milk, precipitation of milk was done to concentrate the protein and the sample was dried at a controlled temperature and powdered to get the desired protein supplement as depicted in the flow chart 1. The selection of fruit was based on the enzyme activity present in fruit samples such as pineapple, papaya and kiwi, cleaned peeled fruit sample was sundried by finely chopping and the powdered fruit sample was stored in an airtight container as shown in the flow chart 2 for further formulation/standardization process.

Standardization

The protein powder was developed with a combination of different legumes and seeds assessed for its flavours. On adjusting various proportions trials were performed to formulate the product with the support of other research trials were performed to standardize the product (Pooja *et al.,*2020).

Sensory Evaluation:

The developed product was formulated with various fruit flavours for its better acceptability and was evaluated by 20 semi-trained panellists at Padmashree Institute of Management and Sciences. The sensory attributes of this product were estimated using the 9-point hedonic scale. The product was examined for its appearance (colour, smoothness), texture (mouth feel), flavour (flavour intensity), taste (taste preference, after taste) and overall acceptability. (Shruthi *et al.,* 2023).

Nutritional composition

The developed samples were analyzed to determine the nutritional composition such as energy content (Food energy analysis & conversion factors: 2003), protein content (AEL/ FDL/SOP/009: 2021), fat content (FSSAI 03.040:2023), carbohydrate content (Food energy analysis & conversion factors: 2003), total dietary fibre (AOAC 985.29), moisture (FSSAI manual cereal & cereal products 03.006:2023) and ash (FSSAI manual cereal & cereal products 03.012:2023).

Confirmatory Test:

The gelatinase test is performed as a qualitative test to confirm the presence of protease activity in the fruit powder. Two test tubes and a glass rod are taken from the biochemistry laboratory at Padmashree Institute of Management and Sciences, Bengaluru. One packet of gelatine was purchased from the nearby supermarket Kengeri, Bengaluru. 1 g of gelatine was taken as control and 0.1g of pineapple fruit powder was weighed and dissolved in the 10ml of boiled water with adjusting consistency (Mekyllah *et al*., 2024). The mixtures in the test tube were stirred and kept in the refrigerator undisturbed and checked every half an hour.

Statistical Analysis

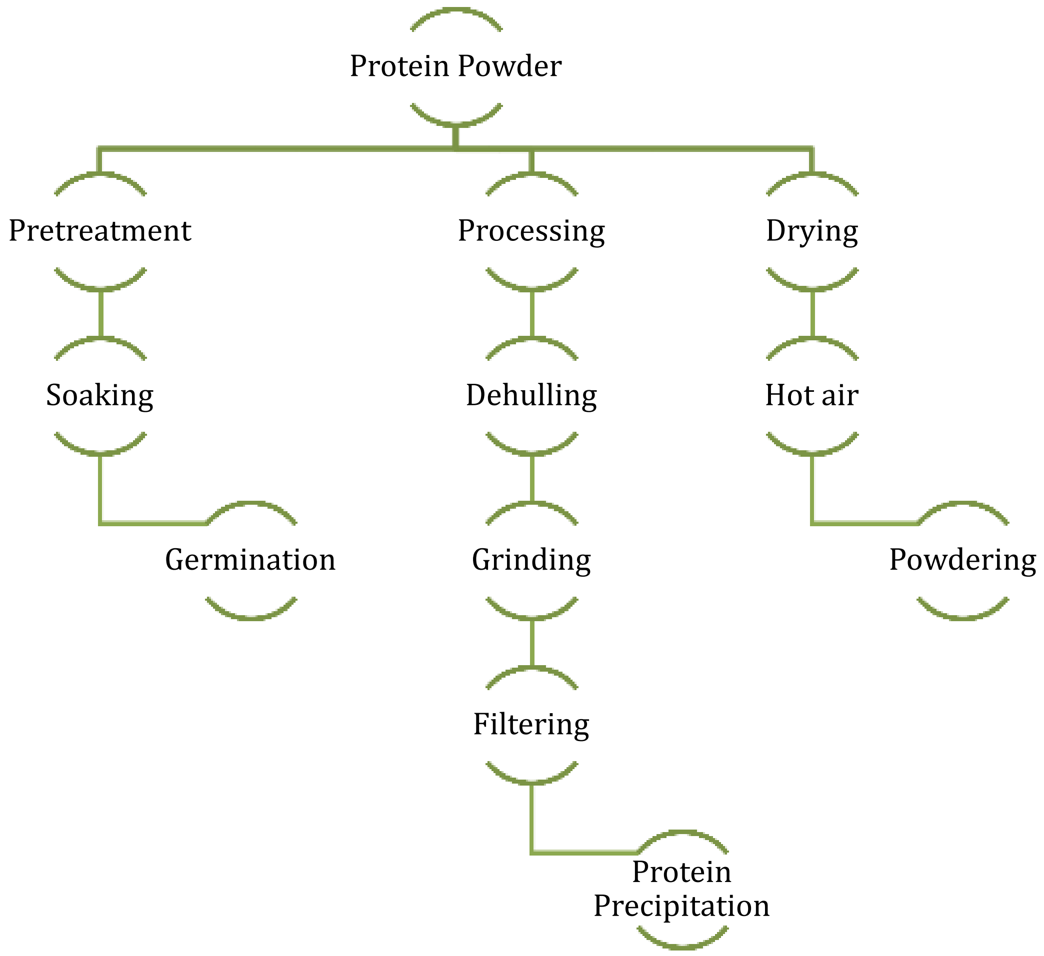
Sensory data were assessed for their average ($\bar{x}$), standard deviation (σ) and F values were compared with $F\alpha$ (ANOVA- single factor) to establish a relation between various fruit flavours and to check means of H_O and H_A.

Table no 1: Composition of existing amino acid profile in grain and seed

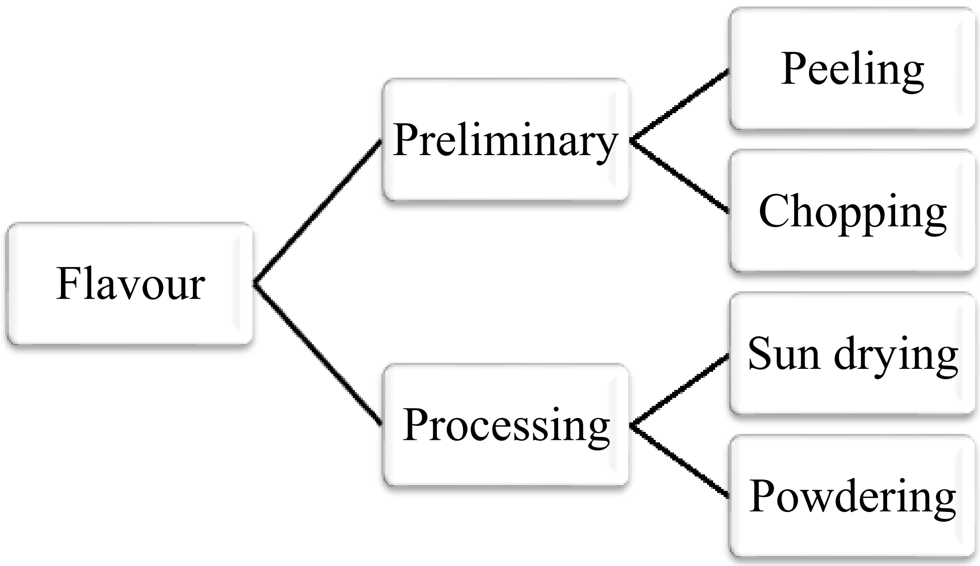
Ingredients	Amino Acids Present
Field Bean	Lysine

	Tyrosine Phenylalanine
Rajmah	All the essential amino acids Aspartic acid
Black Gram	Leucine Lysine Isoleucine Tryptophan Methionine Arginine Histidine Valine Threonine
Amaranth Seed	Histidine Isoleucine Leucine Lysine Methionine Phenylalanine Threonine Tryptophan Valine
Pineapple	Cysteine Methionine Histidine Tyrosine

*Ovidiu *et al*., (2022) *Applied sciences.* *Nowsu *et al*., (2019) *IOSR journal of environmental science, toxicology and food technology.* *Carolina *et al.,* (2023) *Nutrients.* *Marielle *et al*., (2018) *Nutrients.* *Sarkar *et al*., (2023) *Journal of pharmacy and pharmaceutical sciences.*



Flow chart No 1: Research design in processing of protein powder



Flow chart 2: Processing of fruit flavour

Result and discussion

Pre-processing technique

The purchased raw materials such as field beans, rajmah, black gram and amaranth seeds, were soaked in water, among different legumes field beans, rajmah, and black gram had 12 hours whereas amaranth seeds had 24 hours soaking as depticed in table 2. Soaking field beans would increase the density with a higher water absorption, increase porosity and decrease bulk density (Ravikumar *et al*., 2018). Soaking red kidney beans and black gram reduces the phytic acid content, sugars decrease polyphenol and tannin contents (Shimelis *et al.,* 2007; Tripti *et al.,* 2020). Soaking amaranth seeds would decrease moisture and fat content (Priyanka *et al.,*2021).

The germination of red kidney beans and amaranth seeds was 72 hours whereas black gram was 48 hours and field beans 24 hours respectively as depicted table 2; availability of nutrients such as protein, crude fiber, energy, amino acids, calcium, iron, and zinc increases with better antioxidant properties, low moisture and fat content during germination extend the shelf life (Anand *et al.,*2022).

For the protein powder to have better quality the ancient method of pre-processing techniques in legumes i.e., soaking and germination enhances absorption and bulge in size, increasing milk yield for concentrating protein which also improves moisture, antioxidant activity, and nutrient bioavailability making them an excellent vegan protein supplement.

Germination followed by dehulling would change in weight when compared with the raw weight of the sample in field beans (444g), rajmah (455g) and black gram (512g) also improved the protein quality, taste, and digestibility of pulses by decreasing antinutrients. However, the dehulling method had no significant impact on proximate composition but increased the bioavailability of iron and calcium (Oluwole *et al.,* 2011; Reihaneh *et al.,*2007).

The pre-treatment methods were effective in milk extraction, approximately 3000 ml of milk was yielded by combining rajmah, amaranth, field beans, and black gram and was grounded and filtered for better protein precipitation. High heat coagulates the protein and makes it less bioavailable, hence milk with direct contact with heat is an effective method commonly used to deactivate heat-sensitive antinutritional factors, particularly trypsin inhibitors and hemagglutinins with a decrease in starch content (Abbas *et al*.,2018).

Table 2: Soaking and germination duration of selected ingredients

Ingredients	Soaking Duration (hours)	Germination Duration (hours)
Field beans	12	24 hours
Rajmah		72 hours
Black gram		48 hours
Amaranth seeds	24	72 hours

Table 3: Raw, germinated and dehulled weight of selected ingredients.

Ingredients	Raw weight (g)	Germinated weight (g)	Dehulled weight (g)
Field Beans	250	502	444
Rajmah		515	455

Black Gram		555	512
Amaranth Seeds		436	436

Drying legume milk entails eliminating moisture from the liquid to produce a powder. This technique prolongs the milk's shelf life and simplifies its storage and transportation. The process was carried out by placing the sample in a hot air oven, the sample is spread out in plates and kept at 70^0 C for 5 hours cooled at room temperature, yielded about 400g sample

Flavouring

A novel flavor-enhanced protein supplement was developed by incorporating powdered pineapple, kiwi, and papaya into a protein base. The fruits underwent a processing sequence of cutting, chopping, sun drying and fine powder was blended with the protein supplement. Sun drying of these proteolytic fruits takes about 10 days, nearly 8 hours/day. There was a complete shrinkage of fruits and colour changes to dark exhibiting a browning reaction due to the presence of proteolytic enzymes. This resulted in a tropical natural fruit-flavored protein supplement with improved sensory attributes. The resultant flavor weighs accordingly pineapple powder (21g), papaya powder (41g) and kiwi powder (61g). Similar studies revealed that the drying rate increased as the temperature rises and declined with higher humidity. The moisture content dropped from 80% to 20% during drying. The colour change, primarily browning, was caused by enzymatic reactions, and the texture became harder and more brittle, energy consumption for the process was relatively low. Sun drying is an effective preservation method to reduce the moisture content with minimal nutrient losses (Ibrahim,2005)

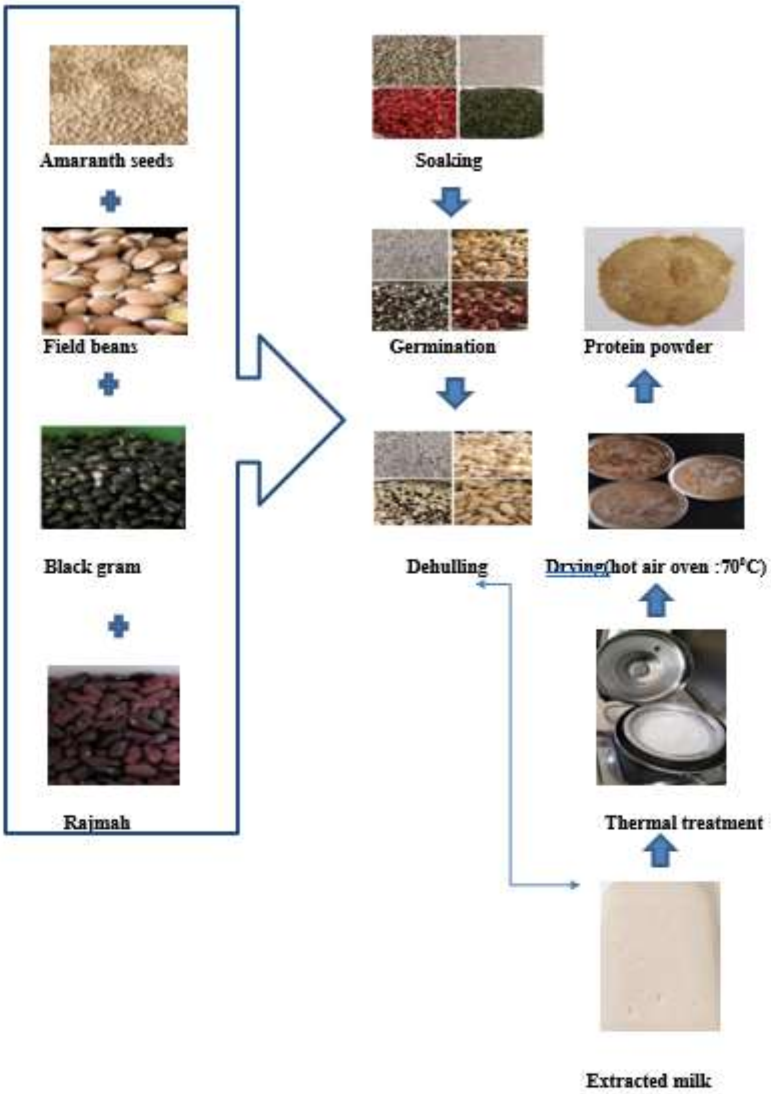
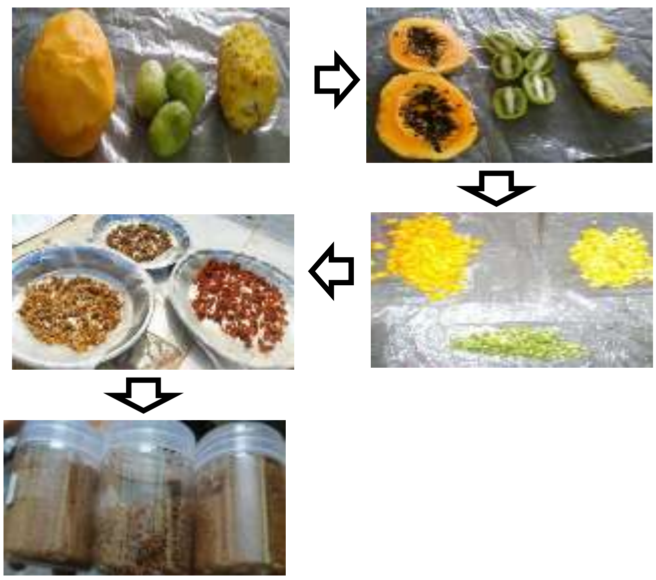


Fig no 1: The process of formulation of protein powder



Flow chart 4: The process of development of flavour.

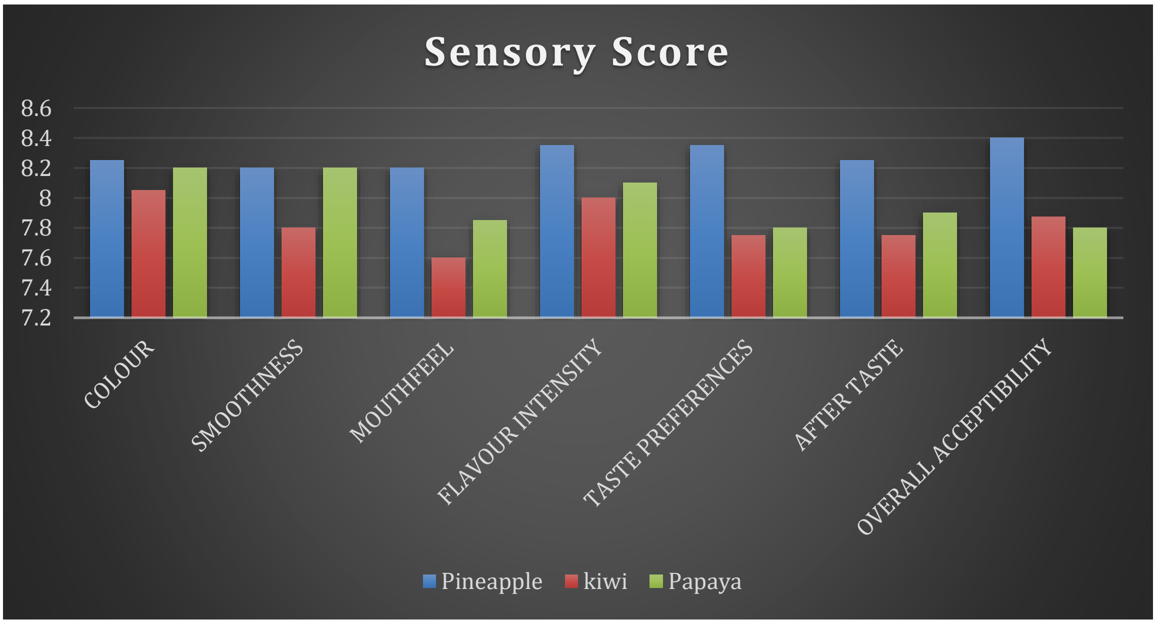
Sensory Evaluation

The sensory evaluation of the developed product included three flavor variations: pineapple (FT_1), kiwi (FT_2), and papaya (FT_3). Among them, FT_1 flavor had the highest acceptability as depicted in table 4. The flavor intensity (8.35 ± 0.74), colour (8.25 ± 0.71), smoothness (8.2 ± 0.76), texture (8.2 ± 0.61), taste preferences (8.35± 0.67) and overall acceptability (8.4 ± 0.59) compared to others, kiwi flavor received slightly lower scores flavor intensity (8 ± 0.85), colour (8.05 ± 0.75), smooth appearance (7.8± 0.89), texture (7.6 ± 1.09), taste preferences (7.75 ±1.01) and overall acceptability (7.875 ± 0.81) .

Table 4: Sensory evaluation of different flavours.

Flavour	Appearance		Texture	Flavour	Taste		Acceptability	
	Colour	Smoothness	Mouthfeel	Intensity	Preferences	After taste	Overall Acceptability	*F value
Pineapple (FT_1)	8.25 ± 0.71	8.2 ± 0.76	8.2 ± 0.61	8.35 ± 0.74	8.35 ± 0.67	8.25 ± 0.78	8.4 ± 0.59	0.25
Kiwi (FT_2)	8.05± 0.75	7.8± 0.89	7.6 ± 1.09	8 ± 0.85	7.75 ±1.01	7.75 ± 0.85	7.875 ± 0.81	0.59
Papaya (FT_3)	8.2 ± 0.76	8.2 ± 0.89	7.85 ± 0.93	8.1± 0.91	7.8± 0.89	7.9± 0.96	7.8±0.95	0.81

*F < Fα [fail to reject (H_0)]



Proximate Analysis

A single legume may lack certain amino acids whereas a blend of multiple legumes called complementary protein would have quality amino acids that can be utilized. Through the processing methods, the quality of protein availability can be enhanced through absorption called bioavailability. The nutritional profile as shown in table 5 of the developed product is impressive, mainly because of its high protein content (26.60g/100g), making it an excellent protein-rich option, especially when compared to other foods. It provides energy (334 kcal/100g) and additionally, it has low-fat (0.38g/100g), carbohydrates (56.05g/100g), dietary fiber (1.71g/ 100g), moisture (13.80 %) and ash (3.17g/100g).

Table 5: Proximate analysis of the developed product.

Sl.No	Nutrient	(g/100g)
1	Energy (kcal)	334.0
2	Fat (g)	0.38
3	Protein (g)	26.60
4	Carbohydrate (g)	56.05
5	Dietary Fibre (g)	1.71
6	Moisture (%)	13.80
7	Ash (g)	3.17

Standardization

Mixture of legume milk powder was standardized with different fruit flavours such as kiwi, papaya, pineapple. Pineapple flavour had overall better sensory attributes. Protein supplement developed can be used by various populations such as athletes, growing children, older people, vegans and diseased patients who require high protein. The size of per scoop is 34 g which gives 9 g of protein. The serving size of standardized scoop includes 3 scoops in 215 ml of milk that gives a dense protein of 27 g.

Amino acid content

In comparing the amino acid content among rajmah, black gram, amaranth, and field beans, notable variations emerge when considering both the averages and standard deviations (SD). Glutamic acid, with an average (μ) 10.5±6.05, shows the highest concentration, particularly in black gram and field beans, indicating its significant contribution to protein. Lysine, with an average (μ) 4.8±6.20, is also abundant, though it varies considerably among ingredients, being highest in rajmah and field beans, as shown in Table 6. Arginine, with an average (μ) 4.7±6.05, stands out prominently, particularly in rajmah. In contrast, amaranth shows the lowest amino acids such as threonine and serine, with averages (μ) 2.6 g and 3 g respectively. Methionine and cystine have the lowest averages ($\mu = 1.1$ g and $\mu = 0.6$ g) and standard deviations ($\sigma = 1.38$ and $\sigma = 0.93$), suggesting they are limiting amino acids. The amino acids isoleucine ($\mu = 4.1$g, $\sigma = 5.71$) and leucine ($\mu = 5.2$g, $\sigma = 6.80$) exhibit considerable variability across the ingredients, with their highest concentrations found in black gram and field beans. In contrast, phenylalanine ($\mu = 4.1$g) and tyrosine ($\mu = 2.3$g). Aspartic acid with an average value (μ) 8.1±10.61, overall field beans and rajmah exhibit richer amino acid profiles, black gram shows greater variability, and amaranth tends to have the lowest concentrations across most amino acids profile.

Table 6: Amino acid profile of selected ingredients

Amino acids (mg)	Rajmah (g)	Black gram (g)	Amaranth (g)	Field beans (g)	Total (g)	Average (g/100)
Lysine	7	6	0.83	5.2	19.03	4.8 ± 6.20
Histidine	3	2.5	0.38	2.59	8.47	2.1 ±2.75
Arginine	6.9	5.2	1.47	5.3	18.87	4.7 ±6.05
Aspartic acid	9.5	12.4	1.22	9.32	32.44	8.1 ±10.61
Threonine	3.4	3.6	0.43	3.04	10.47	2.6 ±3.40
Serine	3.1	4.7	0.88	3.32	12	3 ±3.87
Glutamic acid	10.2	16.2	2.51	13.2	42.11	10.5 ±13.67
Proline	3.3	4.7	0.69	2.85	11.54	2.9 ± 3.76
Glycine	5.2	6.7	1.38	5.15	18.43	4.6 ±5.91
Alanine	4.4	5.7	0.53	4.19	14.82	3.7 ±4.85
Cystine	1.2	Trace	0.19	1.19	2.58	0.6 ±0.93
Valine	4.1	6.6	0.6	5.09	16.39	4.1 ±5.39
Methionine	1.7	0.8	0.34	1.41	4.25	1.1 ±1.38
Isoleucine	3.7	8.7	0.55	3.62	16.57	4.1 ±5.71
Leucine	7.2	5.3	0.86	7.45	20.81	5.2 ±6.80
Tyrosine	3.1	2.5	0.54	3.18	9.32	2.3 ±3.01
Phenylalanine	4.6	5	0.61	6.35	16.56	4.1 ±5.42

*Audu *et al*., (2011) *Pakistan journal of nutrition.* *Shaahu *et al*., (2015) *international journal of scientific and technology research.* *Padhye *et al*.,(1979) *Journal of food science.* *Sylvio et al., (2013) *Food science and technology*

Gelatinase test

The sample was placed in the refrigerator and checked, observation revealed the positive and control samples thickened with gelation, whereas the sample containing fruit powder didn't form gelation as seen in a liquefaction state. The effectiveness of the protease enzyme reaction in the fruit powder is demonstrated by the speed and the duration required to break down the bond to maintain an ungelatinized state (Mekyllah *et al*., 2024). This confirms the presence of protease in the fruit powder prepared. Due to the presence of protease enzyme in the fruit powder, the enzyme digests the gelatin not allowing it to form gel resulting in the sample remaining liquid, as presented in the fig no 2. Demonstrates the enzymatic reaction between bromelain and gelatin, showcasing the impact of enzyme reaction on protein structure and function. Bromelain breaks down gelatin's proteins by cleaving peptide bonds, leading to the degradation of its protein structure and loss of gel-forming ability.

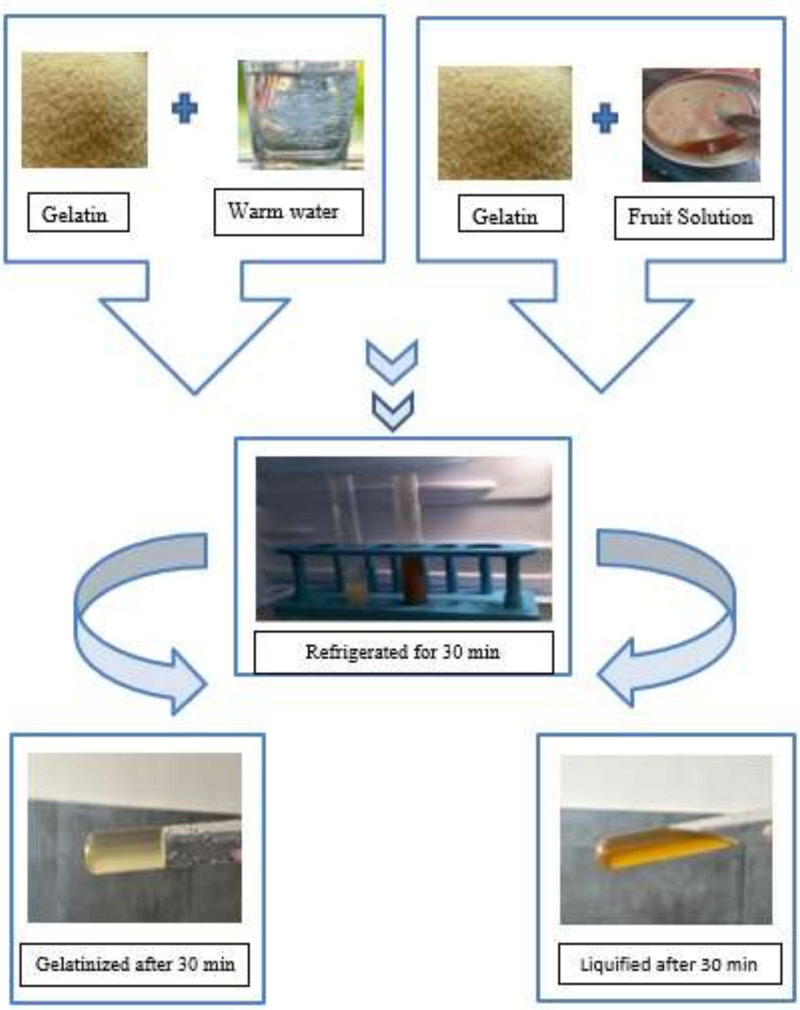


Fig no 2: Confirmatory result of gelatin test

Statistical Analysis

In the sensory evaluation of different flavors (pineapple, kiwi, and papaya), various attributes such as appearance, texture, flavor, taste, and overall acceptability were assessed. All the flavors are not significant f-values and p-values obtained for pineapple, kiwi, and papaya, were 0.25, 0.59, 0.81 and 0.9, 0.59, 0.56 respectively. All the F values < F α (2.1674) and P value > α (0.05) therefore sensory study failed to reject H_0, which indicates that all flavors are significant. The data suggests that the flavors may have significant effects.

Conclusion

The developed amino acid rich plant-based protein supplement using legumes, seeds, and proteolytic fruits was created with an optimized amino acid profile, enhanced digestibility, and appealing natural flavors. In terms of practical applications, this protein powder can cater to a wide range of consumers looking for sustainable, plant-based alternatives to dairy or animal-derived protein supplements. The product's low-fat and high-protein content make it an attractive option for those seeking to enhance their protein intake without added fats or sugars. The use of plant-derived ingredients and traditional processing methods reduces environmental impact while offering a highly nutritious product with null food additives. Additionally, the relatively small scale of sensory evaluation and limited shelf-life studies means that larger-scale testing is required before full commercialization. The enzyme activity from fruits also requires further investigation to determine how long they remain active in the final product during storage. Future research could focus on optimizing the drying process to preserve the nutritional value of the fruit components and conducting more extensive consumer studies to assess market acceptability. Also offers a promising foundation for the future development of innovative, sustainable protein products that can meet the needs of health-conscious consumers and those following plant-based diets.

References

1. Alberta N. A. Aryee., Joyce I. Boye., "Improving the Digestibility of Lentil Flours and Protein Isolate and Characterization of Their Enzymatically Prepared Hydrolysates", International Journal of Food Properties, 2016, 19.
2. Andrea K. Stone., Matthew G. Nosworthy., Constance Chiremba., James D., Michael T. Nickerson., "A comparative study of the functionality and protein quality of a variety of legume and cereal flours", Ceral and grain association, Cereal Chemistry, 2019,1(11).
3. AOAC Official Method 985.29, Total Dietary Fiber in Foods Enzymatic-Gravimetric Method.
4. Bhakthi Anand Narale., Akalya Shanmugam., Ashish Rawson., "Effect of pre-treatment of field bean for enhancement of protein content", The Pharma Innovation Journal, 2022,11(10), 550-554.
5. Carolina Anazco., Paola G. Ojeda., Marion Guerrero Wyss., "Common Beans as a Source of Amino Acids and Cofactors for Collagen Biosynthesis", Nutrients, 2023, 15(21): 4561.
6. Carolina Varilla., Massimo Marcone., "Bromelain a Group of Pineapple Proteolytic Complex Enzymes (*Ananas comosus*) and Their Possible Therapeutic and Clinical Effects", A Summary Foods, 2021, 10 (10).
7. Chad M. Kerksick., Christopher J. Rasmussen., Stacy L. Lancaster., Bharat Magu., Penny Smith., Charles Melton., Michael Greenwood., Anthony., Almada., Conrad P. Earnest., Richard B. Kreider., "The effect of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training," Journal of Strength and Conditioning Research, 2006, 20(3), 643–653.
8. Chockry. Barbana., Joyce Irene Boye., "In vitro protein digestibility and physico- chemical properties of flours and protein concentrates from two varieties of lentil (Lens culinaris)", Food & Function, 2013, 4 (310).
9. Dr. Pooja Maurya., Akanksha., "Standardization and development of Beet root powder incorporated food products with their quality evaluation", Journal of Pharmacognosy and Phytochemistry, 2020, 9(4): 3212-3217.
10. Food energy- methods of analysis & conversion factors, FAO Food and Nutrition paper, 2003,77; 7-11.
11. Food energy- methods of analysis & conversion factors, FAO food and nutrition paper, 2003,77. 12-17.
12. Food energy methods of analysis & conversion factors, FAO Food and Nutrition paper, 2003, 77. 18-60.
13. FSSAI 03.040:2023, Determination of Oil/Fat by Soxhlet Extraction;106-107.
14. FSSAI manual cereal & cereal products 03.006:2023, Determination of Moisture in Cereals and Cereal Products; 22-23.
15. FSSAI manual cereal & cereal products 03.012:2023, Determination of Acid Insoluble Ash; 34-35.

16. Ibrahim Doymaz., "Sun drying of figs: an experimental study, journal of food engineering",2005,71(4);403-407.
17. Ma. Mekyllah B. Julio., Eric M. Solera., Patrick Ian John N. Ferolino., Allan Ismael D. Redecio., "An enzyme at work: liquifying gelatin with pineapple", 2024 January.
18. Marielle Margier., Stephane George., Noureddine Hafnaoui., Didier Remond., Marion Nowicki., Laure Du Chaffaut., Marie-Josèphe Amiot., Emmanuelle Reboul., "Nutritional Composition and Bioactive Content of Legumes: Characterization of Pulses Frequently Consumed in France and Effect of the Cooking Method Nutrients", 2018, 10(11).
19. N. Ravikumar., Roopa R S., Udaykumar Nidoni., C T Ramachandra,K.V., Prakash,H. Sharanagouda., "Engineering Properties of Field Beans (Lablab purpureas L.) at Different Soaking Time", International journal of current microbiology and applied sciences, 2018, 7(3):2024-2036.
20. Nwosu, J.N., Anyaehie, M.A. Ofoedu, C.E., "Effect of Different Processing Techniques on the Amino Acid Profile of Black Gram", IOSR Journal of Environmental Science, Toxicology and Food Technology, 2019,11(13),79-84.
21. Oluwole Akinjayeju., Olayinka. F Ajayi., "Effects of dehulling on functional and sensory properties of flours from black beans (phaseolus vulgaris) -properties of black beans flours", Food and Nutrition Sciences, 2011, 2(4).
22. Ovidiu Procopet., Mircea Oroian., "Amaranth seeds polyphenol, fatty acid and amino acid profile", Applied sciences, 2022 ,12(4).
23. Priyanka Thakur., Krishan Kumar., Naseer Ahmed., Divya Chauchan., Qurat U.I Eain Hyder Rizvi., Sumaira Jan., Tajendra Pal Singh., Haracharan Singh Dhaliwal., "Effect of soaking and germination treatments on nutritional, anti-nutritional, and bioactive properties of amaranth, quinoa and buckwheat", Current research in Food Science. 2021, 4: 917-925.
24. Ravi Shankar., Prabhat Kumar Upadhyay., Manish Kumar., "Protease enzyme: Highlights on Potential of proteases as Therapeutic Agents", International Journal of peptide research and therapeutics, 2021.
25. Reihaneh Ahmadzadeh Ghavidel., Jamuna Prakash., "The impact of germination and dehulling on nutrients antinutrients in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seed", LWT, 2007, 40(7): 1292-1299.
26. Saratu Stephen Audu., Mathew Olaleke Aremu., "Effect of Processing on Chemical composition of Red Kidney Bean (Phaseous Vulgaris L.) flour". Pakistan Journal of Nutrition, 2011, 10(11)
27. Sarkar S., Mandal S., Sen D., Das N., Mondal T., Paul A., Chakrabarty S., Barik B., "Identification of Amino Acid and Proteolytic Activity on Protein by Pineapple (Ananas Comosus) Enzyme Extract", Journal of Pharmacy and Pharmaceutical Sciences, 2023, 1(12).
28. Shaahu, D.T., kaankuka, F.G., Okpanachi,U., "Proximate, amino acid, anti-nutritional factor and mineral composition of different varieties of raw lablab purpureus seeds", International journal of scientific and technology research. 2015, 4(4).
29. Shimelis Admassu Emire., Sudip Kumar Rakshit., "Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (Phaseolus vulgaris L.) varieties grown in East Africa". Food chemistry. 2007, 103(1).
30. Shruti., Pavan Kumar., Suresha KB., GS Krishna Reddy., "Study the effect and properties of spray dried grain amaranth malted milk powder", The Pharma Innovation Journal 2023, 12(11): 1831-1837.
31. Sylvio Vicentin Palombine., Thiago Claus., Swami Arta Maruyama., Aline Kirie Gohara., Alonio Henrique Pereira Souza., Nalson Evdazio D.E Souza., Jesui Vergilio Visentainer., Sandra Terezinha

Marques Gomes., Makoto Matsushita. "Evaluation of nutritional compounds in new amaranth and quinoa cultivars. Food science and technology", 2013, 33(2): 339-344.

32. Tripti Ranabhat., "Effects of processing techniques on anti-nutritional factors of black gram (*vigna mungo*)", 2020
33. V.W. Padhye., D.K. Salunkhe., "Biochemical studies on black gram (phaseolus mungo l.) seeds: amino acid composition and subunit constitution of fractions of the proteins", Journal of Food Science, 1979, 44(2).
34. Yasir Abbas., Asif Ahmad., "Impact of processing on nutritional and antinutritional factors of legumes", Food science and Technology, 2018, 19(2).
35. Yohan Reynauda. B., Michel Lopeza., Alain Riaublancc., Isabelle Souchond., Didier Dupont., "Hydrolysis of plant proteins at the molecular and supra-molecular scales during in vitro digestion", Food Research International. 2020, 134.