

# Formulation & evaluation of plant-based RTU protein mix for athletes

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**Abstract.** Protein is considered as a key component for the athletes and fitness adherent for their performance enhancement. Athletes chose the animal-based proteins are the source of protein where the bioavailability and digestibility are good, but animal-based protein contains more saturated fat where it leads to the many chronic diseases and also improves weight. To produce meat as protein source from animals it takes more land, water and other resources when compared to the plant-based proteins. The online survey done on the commercial protein mixes it was found that a few cost-effective plant-based protein mixes are available for the Indian fitness adherents and athletes. It was also observed that most of the female athletes and fitness adherent are not meeting the RDA of essential nutrients like iron and calcium which results in low nutritional status and reduced recovery rates. In the present study a sustainable plant-based protein mix was developed which may enhance the athletic performance especially in female athletes by improving muscle mass, muscle recovery, weight management and meeting RDA. The developed mix showed good nutritional profile along with the presence of the essential amino acids and a moderate to high digestibility score of 0.70 in terms of PDCAAS. The mix also exhibited significant ( $p < 0.05$ ) anti-oxidant and anti-inflammatory properties. The developed mix was also studied for other properties such as water absorption capacity, hygroscopicity, solubility index, bulk density, tapped density and also the swelling index. The solubility index and swelling index were  $79.2 \pm 1.13$  and  $4.35 \pm 0.21$ . The product also showed good sensory scores ranging from 7-8 out of 9-point hedonic scale along with shelf stability of 4 months.

KEYWORDS: Plant-based proteins, athletes, fitness, sustainable

## 1. INTRODUCTION

During strenuous physical activity and in energy metabolism many micronutrients plays a crucial role. During this kind of activity, the energy turnover rate in skeletal (involuntary) muscles increases up to 20-100 times than during relaxation phase. Athletes have higher requirement of macro and micronutrients they have to consider the intake of minerals such as iron and calcium and vitamins with antioxidant potential during their hard training [1].

Most of the female athletes are facing many health problems due to inadequate dietary intake that leads to the lack of supply of enough nutrients to the body to support their physical demands of training. They are also at the risk of mineral deficiency especially calcium and iron that results in the amenorrhea, abnormal eating pattern, osteoporosis. Fuelling of athletes is crucial to enhance

their performance and endurance level by providing a diet rich macro and micronutrients [2]. Protein powders and other meal replacements are the various ways where female athletes can fulfil their protein supplements.

Minerals are required for different metabolic and physical processes in a living system. They have important roles such as heart rhythm, conducting nerve impulses, maintaining healthy immune and skeletal system and muscle contraction. An adequate amount of minerals is essential for optimal functioning during exercise. Iron and calcium are the two micronutrients most likely to be low particularly in the diets of young athletes [3].

Due to high digestibility factor and biological value of plant-based isolates like pea protein and brown rice helps in providing a good quality of protein and contain almost all the required amino

acids. Athletes prefer these in the sports beverages as a replacement of whey protein isolates [4]. They help in the postprandial muscle protein synthesis, repair the damaged tissues and enhances energy production.

Protein intake was suboptimal in Indian competitive runner’s boxers and weight lifters. North Indian female hockey players, volley ball player and runner negative energy balance was observed in the lives of state or national level sports women and female hockey players. Low carbohydrate intake and higher intake of fat was observed to be the recommendation in female kabaddi [5].

Calcium deficiency leads to micro architectural deterioration that may develop into premature osteoporosis, and in severe condition leads to an anterior cruciate ligament (ACL) and stress fractures [6]. Metabolic disorders like diabetes and thyroid problems will profoundly affect the athletic performance. Sleep disturbance is common problems in athletes and they also suffer runners diarrhoea and is usually seen in distance runners. Reduced LDL and hypercholesteromia

were reported by DE Matos et al. (2011) in south Indian professional weightlifters. [7] also reported high prevalence of blood pressure in male and female athletes along with low ferritin and anaemia in female athletes and fitness adherents.

Plant-based diets and proteins as performance enhancers Plant based diets improve or escalate athletic performance as they are high in protein and carbs, vitamins, minerals and antioxidants and low in fat. Athletes can meet their calorie and nutrient requirements with the various types of vegetarian diets. This plant based also helps in reducing chronic diseases [8] and enhances their ability to perform optimally or recover from strenuous exercise [9]. Fig. 3 depicts Positive and negative interactions of PBD on exercise performance.

**Table 1. Potential risk of nutrient inadequacies**

Nutritional components	functions	Impact during inadequacy	references
Proteins & amino acids	Muscle function, fatigue, recovery in exercise	Reduces muscle and integrity, hematopoiesis	(Ohtani, 2023)
n-3 & n-6 fatty acids	Reduce inflammation, atherogenic, prothrombotic	Increases membrane fluidity	(gerd, 2007)
Iron	Energy metabolism, oxygen transport, acid- base balance	Fatigue, weakness, reduce muscle strength	(Adam,2023)
Zinc	Improves lean muscle mass & strength, boost immune system, improves insulin sensitivity		(drew, 2014)
Vitamin B12	Improves performance, enhanced recovery, improves energy production, improves oxygen delivery	Fatigue, anaemia, muscle weakness, nerve damage and mood disturbances, tingling sensation in hands and feet	(Krzywanski,2022)
Vitamin D	Bone health, muscle function, inflammation and immunity	Bone injury, stress fractures, impair muscle strength and performance	(Phillip B.Wyatt,2024)
Calcium		Reduces muscle contraction and bone density leads to osteoporosis, Osteomalacia, bone resorption	(Hadeel Ali Ghazzawi, 2023)

Due to high digestibility factor and biological value of plant-based isolates like pea protein and brown rice helps in providing a good quality of protein and contain almost all the EAA. They are most preferred in the sports beverage considered

as a replacement of whey protein [8]. They help in the postprandial muscle protein synthesis, repair the damaged tissues and enhances energy production.

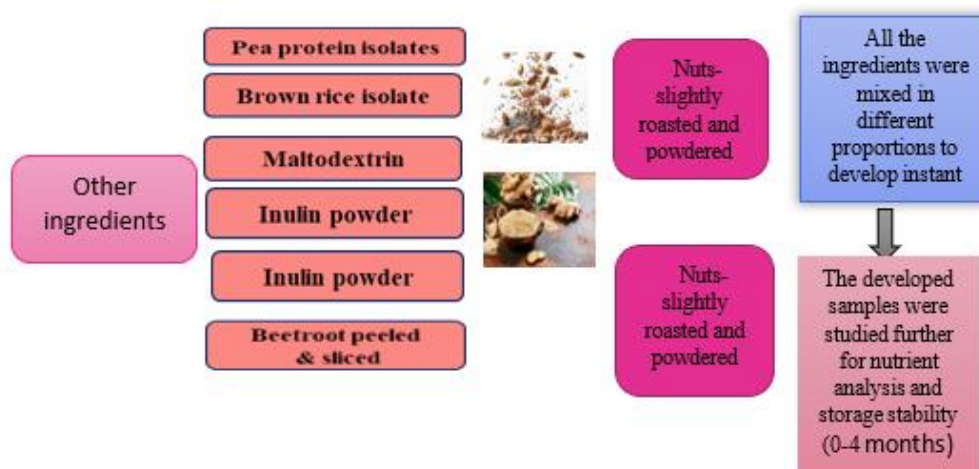
## 2. MATERIALS AND METHODS

The current study has been carried out to investigate the nutritional and phytochemical profile of the developed product along with in vitro therapeutic potential using standard protocols

### 2.1 Formulation and standardization of the products

Vegetables and green leaves were cleaned and washed fig. 4 presents the processing and preparation of the instant mixes to remove any foreign matter if present, dried at 45°C for 4-12 hours using table top lab dehydrator (Ezidri Ultra FD1000 model) followed by coarse powdering. The nuts and spices were slightly roasted and powdered.

**Fig: 1 Processing and preparation of plant- based protein mix**



### 2.2 Preparation and reconstitution of the developed product

**Plant based protein mix** The ingredients were weighed and mixed together. To this mixture, other ingredients such as vegetables, spices, nuts,

and oilseeds mix were added. The dry mix was blended well to a fine powder using a normal kitchen blender. The developed product of the mixes was reconstituted with measured amount of water, milk stirred and were evaluated for their acceptability.

### 2.3 Physical properties analysed in the developed product

The physical properties such as bulk density, tapped density, flowability, cohesiveness, swelling index, hygroscopicity and water solubility index were measured.

#### Bulk and tapped density

A measured amount of sample was placed into a 10 mL measuring cylinder, and the volume occupied by the powder was measured and subsequently used to determine the bulk density (weight per unit volume).

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Mass of the powder}}{\text{Initial volume of the powder}}$$

The tapped density was calculated by tapping the measuring cylinder for 5 min until no visible decrease in volume was noticed. The final volume was then read and used to calculate the tapped density.

$$\text{Tapped density (g/cm}^3\text{)} = \frac{\text{Mass of the powder}}{\text{Final volume of the powder}}$$

#### Flowability and cohesiveness (%)

The flowability and cohesiveness of sample is provided in terms of Carr index (CI) and Hausner ratio (HR), respectively. CI and HR are calculated based on the tapped density ( $\rho_T$ ) and bulk density ( $\rho_B$ ) of the powder as per the below mentioned formulas.

$$\text{Carr index (CI) (\%)} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

#### Swelling index (SI)

The swelling index of sample was determined based on a previously reported method. 1.0 g of sample was weighed into measuring cylinder (10 mL) and the distilled water (5 mL) was carefully added, then the volume occupied by the sample was recorded. The sample was allowed to stand undisturbed in water for 1 h and the volume occupied after swelling was recorded. The

swelling index was calculated using the following formula.

$$\text{Swelling index (\%)} = \frac{\text{Volume occupied by sample before swelling}}{\text{Volume occupied by sample after swelling}} \times 100$$

#### Hygroscopicity

Hygroscopicity of sample was calculated according to the previous method with slight modifications. Approximately 1.0 g of sample was placed in petri dishes in a glass desiccator containing saturated NaCl solution and stored for 7 days at 25 °C.

$$\text{Hygroscopicity (\%)} = \frac{\text{Weight gain by powder after 7 days (g)}}{\text{Weight of sample (g)}} \times 100$$

#### Water solubility index (WSI)

Water solubility index (WSI) of the sample was determined using shake-flask method Sample (0.5 g) was suspended in 50 mL distilled water and the suspension was incubated at 60 °C for 1 h. The suspension was centrifuged at 1431 × g 30 min, the insoluble residue was recovered and dried at 60 °C until constant weight was achieved. WSI (%) was calculated using following formula.

$$\text{WSI (\%)} = \frac{\text{Insoluble residue weight (g)}}{\text{Original sample weight (g)}} \times 100$$

## 3. RESULTS & DISCUSSION

Instant mix was developed using pea and brown rice protein isolate as the primary ingredients. The isolates have gastric quick emptying time, help in muscle recovery, maintain ideal body weight, enhances stamina, stabilizes blood sugar levels and re also known for acting as anti-oxidants, anti-inflammatory and immunomodulation agents (Ding-Tao Wu, 2023). Beetroot was added as it is rich source of calcium and iron, which aids in increasing the haemoglobin levels and maintain good bone health. It is rich in ( $\text{NO}_3^-$ ) content which works as a performance enhancer by sparing oxygen and improving exercise tolerance. Ginger and cinnamon act as anti-inflammatory agents helps in decreasing muscle soreness,

decreases inflammation, oxidative stress, and muscle damage at post exercise [2] Nuts such as almonds, pistachio and oilseeds like sesame seeds and flax seeds were roasted, ground and mixed together and added as a source of polyunsaturated fatty acids (PUFA). Flaxseeds characterizes a rich source of PUFAs (e.g.,  $\alpha$ -linolenic acid), which have been positively associated with a

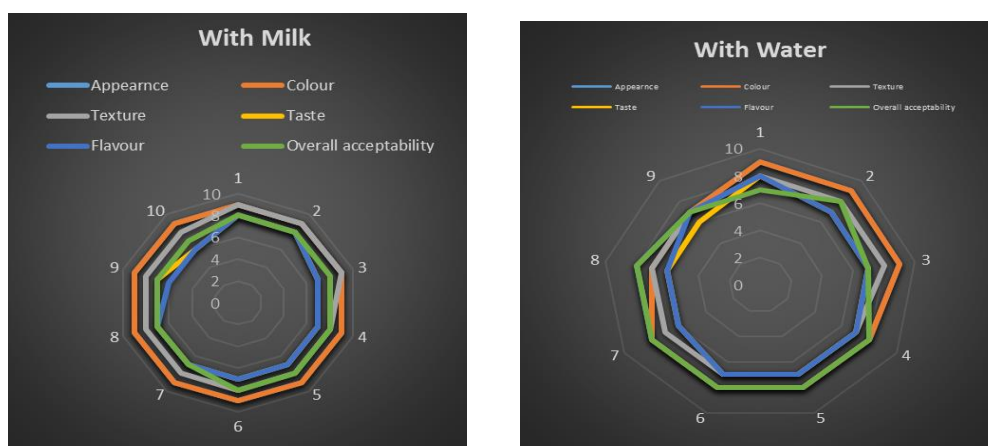
variety of health benefits, like protection against inflammation.

The developed protein mix was reconstituted with water (200ml) and milk (200ml). Sensory evaluation was carried out for sensory parameters like appearance, colour, taste, constituency, flavour and overall acceptability with the sensory score of 9-point Hedonic scale. The most preferred was protein mix with milk

**Table 2 (a). Sensory score of the milk reconstituted developed product**

Sensory parameters	Developed mix	Commercial mix
Appearance	9±0.0	7.67±0.85
Color	9±0.0	7.5±0.87
Taste	7.1±0.56	6.85±0.64
Flavor	7±0.6	6.67±0.64
Texture	8.3±0.4	6.92±0.95
Overall acceptability	8.0±0.51	7±0.76

Values are mean ±SD mean of 10 replicates



**Fig: 2(a) & (b) Sensory analysis of the developed product**

**Table 2 (b). Sensory score of the water reconstituted developed product**

Sensory parameters	Developed mix	Commercial mix
Appearance	8.2±0.67	7.75±0.72
Color	8.1±0.70	7.67±0.85
Taste	7±0.60	7.83±0.69
Flavor	7±0.60	7.83±0.80
Texture	7.3±0.50	7.5±0.76
Overall acceptability	8.0±0.52	7.75±0.60

Values are mean ±SD mean of 10 replicates

### 3.1 Proximate analysis

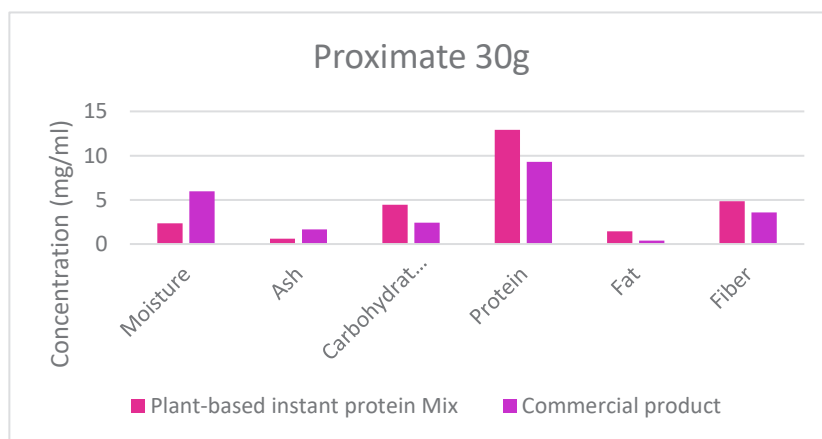
The developed product was found to be high in protein content than the commercial product. The fat content of the developed product and commercial product was found to be 3.45 and 1.35 g/100g respectively. The commercial protein mix showed very low fat content of 1.35. the fiber content of the developed product was found to be slightly high of 12.89 when compared with the

commercial product of 11.9 g/100g. Since we could not undertake amino acid analysis in the developed product, amino acid composition of the product was calculated using standard values taken from [9] Essential amino acids have the potential to stimulate muscle protein synthesis. All the essential amino acids were found in the developed product total of 13.25 mg/100g and highest is the leucine of 7.48. Non-essential amino-acids was found to be 10.24 mg/100g.

**Table 3. Proximate analysis of the developed product per serving(g/30g)**

Sample	Moisture	Ash	Carbohydrates	Protein	Fat	Fiber
<b>Plant-based instant protein Mix</b>	2.35±0.17	0.59±0.31	4.45±0.05	12.93±1.0	1.44±0.00	4.857±0.00
<b>Commercial product</b>	5.97±1.62	1.641±0.06	2.4±1.0	9.3±1.09	0.40±0.18	3.57±0.08

Values are mean ±SD mean of 10 replicates



**Fig.3 Proximate analysis of the developed product (g/30g)**

### 3.1.2 Protein Digestibility Corrected amino acid score (PDCAAS)

The pea protein digestibility was found to be around 97.3% whereas the pea protein estimated PDCAAS is 93% for adults (WHO, 2007). Also this protein

has similar quality to that of casein and eggs than the other plant sources. The developed product is exhibiting the moderate to high PDCAAS value of 0.68.

### 3.1.3 Minerals

The developed product was analysed for the mineral content (Calcium, iron and phosphorous). The calcium content was found to be highest in the developed product (103 mg) than the commercial product (32). Calcium plays an

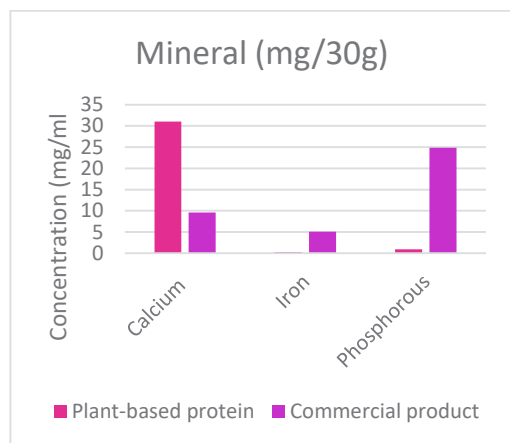
important role in an athlete’s health, training, and performance [10].

The iron content of the developed product is 0.87 whereas the commercial product is exhibiting the extremely high levels of iron 17 which could be due to the fortification of the mix.

**Table 4. Mineral content of the developed product**

Samples	Minerals (mg/100g)		
	Calcium (mg/30g)	Iron (mg/30g)	Phosphorous (mg/30g)
Plant-based protein mix	31.05±0.18	0.26±0.00	0.94±0.04
Commercial protein mix	9.6±1.5	5.1±0.21	24.9±0.23

Values are mean ±SD mean of 10 replicates



**Fig. 4 Mineral content of the developed product (mg/30g)**

### 3.2 Phytochemical profile

Phytochemicals such as anthocyanin’s, flavonoids, polyphenols, vitamin C were analyzed in the developed product (Table 5). Polyphenols have a wide range of biological activities which act as anti-oxidant, anti-carcinogenic and anti-mutagenic properties and possess the ability to modify gene expression. Anthocyanin also acts as an anti-oxidant which provide several health benefits include potential protection against the type 2 diabetes, cancer, heart disease and cognitive health decline. The total phenol content (mg/100g on dry weight basis) was found to be (543) and least in commercial product. The developed product exhibit high levels of polyphenols due to incorporation of functional ingredients such as protein isolates, dehydrated vegetables and flower. The developed mix showed content of 6.3, 9.3 and 8.68 mg/100g on dry weight basis for anthocyanin’s, flavonoids and vitamin C respectively.

**Table 5. Phytochemical content (mg/100g) of the developed product**

Sample	Anthocyanin (mg/100g)	(mg/30g)	Flavonoids (mg/100g)	(mg/30g)	Phenols (mg/100g)	(mg/30g)	Vitamin c (mg/100g)	(mg/300g)
Plant-based instant protein Mix	6.3±0.3	1.89±0.1	9.3±0.1	2.79±0.01	543±0.8	162.9±0.28	8.68±0.05	2.60±0.00
Commercial product	--		22.6±0.00	6.8±0.05	147±0.00	44.1±0.2	111±1..0	33.3±0.01

### 3.3 Therapeutic assessment of the developed products using in vitro protocols

The developed products were analysed for their therapeutic potential in terms of anti-oxidant and

anti-inflammatory potential using standard in vitro protocols.

### 3.3.1 Anti-oxidant activity

The anti-oxidant activity potential was assessed in metabolic extracts of the developed products using the following assays: ferric reducing antioxidant power (FRAP), and reducing power. These activities were compared with quercetin or butylated hydroxytoluene as the standards.

#### Reducing power

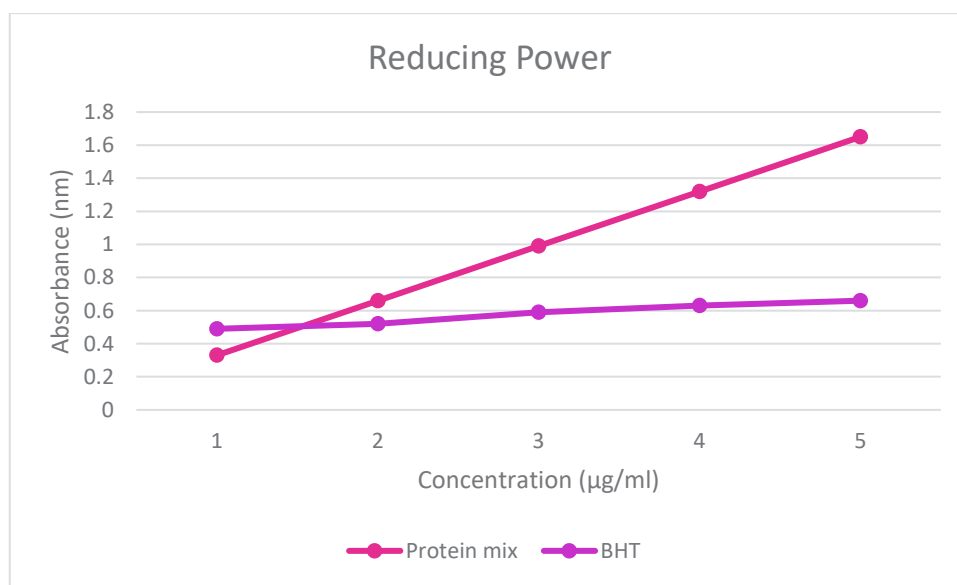
The sample extracts exhibited higher reducing power. At 50µg/ml concentration of the extracts,

the reducing power of plant based protein mix was found to be maximum (1.65). Reducing power exhibited by the standard BHT (0.66) was found comparable to the results revealed by the methanolic extracts (fig. 5). The reducing ability of a sample is associated with the presence of reductones possessing hydrogen donating ability. These active compounds show anti-oxidant activity by breaking the free radical chain reactions. In the present study, the results obtained could be due to the presence of reductone compounds such as phenolic acids and flavonoids.

**Table 6. Reducing power of the developed product**

Samples	Concentration 100 mcg/ml				
	10	20	30	40	50
Plant based protein	0.33±0.04	0.66±0.01	0.99±0.04	1.32±0.01	1.65±0.04
BHT	0.49±0.01	0.52±0.02	0.59±0.02	0.63±0.05	0.66±0.02

Values are mean± SD of three replicates on dry weight basis



**Fig. 5 Reducing power of the developed product**

### 3.3.2 Anti-inflammatory activity

Inflammation is generally referred to as a complex biological response of vascular tissues to harmful stimuli. As well, inflammation is associated with pain, and it involves in an increase of protein denaturation, an increase of vascular permeability, and membrane alteration, among others. It is one of the major problems that athletes come across on daily basis majorly due to exercise-induced muscle damage. Reducing

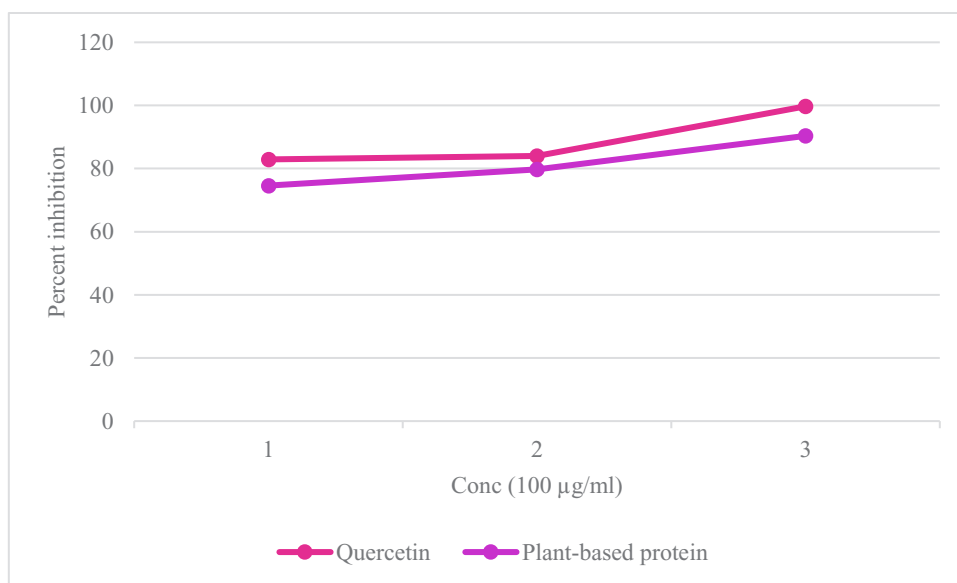
inflammation and quick recovery from damage is an essential for athletes to have better performance. These are various synthetic steroidal and non-steroidal anti-inflammatory agents that are in use by the athletes. Some of the major plant-derived bioactive compounds which have been identified with inflammation and pain relieving properties include curcumin, resveratrol, capsaicin, epigallocatechin-3-gallate, and quercetin. Since we have developed products for the athletes, we wanted to study the impact of the

products as anti-inflammatory agents against protease using in vitro standard protocols.

**Table 7. Protease inhibitory action**

Samples	Concentration 100 mcg/ml		
	2.5	5	10
Quercetin	82.91±0.00	84.01±0.01	99.72±0.16
Plant-based protein mix	74.61±0.06	79.72±0.13	90.36±0.05

Values are mean± SD of three replicates on dry weight basis



**Fig.6 Protease inhibitory action of the developed product**

The Protein mix showed the maximum inhibition of (90.36). The standard quercetin showed the maximum inhibition of (99.72) at 10µg/ml. The results of this study also showed that there is a significant correlation between the studied anti-inflammatory properties and their estimated total

polyphenol, flavonoids and vitamin C. the present indicates that the evaluated anti-inflammatory properties may be related to the presence of antioxidant bioactive, such as polyphenols, flavonoids, and carotenoids.

**3.4 Storage stability of the developed Protein mix**

In order to assess the effect of storage on the shelf life of the developed products, they were stored in

aluminium pouches for a period of 4months under ambient temperatures. The stability of the samples was assessed by estimating the pH, colour, water activity at regular intervals of 2months i.e., 0, 2, & 4months.

**Table 8. Impact of storage on pH, TA and water activity of the developed product**

Property	0 Month	2 <sup>nd</sup> Month	4 <sup>th</sup> Month
pH	4.86±0.00	5.0±0.00	5.34±0.00
Titrate acidity	2.4±0.00	3.04±0.00	3.2±0.1
Water activity	0.50±0.00	0.53±0.005	0.55±0.01

Table 8 presents the impact of storage on the colour of the Protein mix. Colour is an important aspect, which determines the aesthetic quality of food to the customers. In the current study, L, a, and b values of plant-based Protein mix, were measured. (L) indicates lightness, (a) corresponds to greenness or redness of the sample and (b) corresponds to blueness or yellowness of the sample. With the passage of time, the lightness (L) of the products decreased slightly. The (a) value

has slightly decreased for plant based Protein mix. The (b) value has decreased for the plant based Protein mix over 4 months.

The impact of the storage on the overall acceptability of the developed products was observed. The samples were tested for any changes in the flavour, taste, consistency/texture, appearance, colour and overall acceptability. The samples were found to be stable over a period of 4months when stored at right temperature in aluminium pouches.

**Table 9. Impact of storage on colour of developed product**

Sample	L			a			b		
	Month								
Plant-based protein mix	0	2 <sup>nd</sup>	4 <sup>th</sup>	0	2 <sup>nd</sup>	4 <sup>th</sup>	0	2 <sup>nd</sup>	4 <sup>th</sup>
		50.9±1.1	51.5±0.9	51.7±1.0	-0.4±0.05	-0.6±0.1	-0.7±0.1	6.4±0.05	6.86±0.06

### 3.5 Physical properties of the developed product

When the bulk density is less it is nutritionally beneficial and also it helps in promoting the digestibility of the product. When the bulk density is higher the denser will be the packaging material. When the bulk density is high it helps in reducing the transportation cost as well as the storage.

Hygroscopicity of 10.46 indicate the control of moisture. Carr's index of 40.32 measures the compressibility of the powder which indicates how much

powder can compact. Swelling index of 4.35 indicates its ability to swell and potential impact on digestion or release of ingredients. The flowability of the powder will also be determined by the hygroscopicity of the product. The size of the particle effects the swelling capacity. The lower value of carrs index determines the good flowing property. The developed product is exhibiting the 40.32 carrs index. The consistency & flow properties of the powder can be determined by the cohesiveness of the powder. Where lower the cohesiveness

better will be the flow property. The range from lower cohesiveness is  $\leq 1.2$  (low cohesiveness) and 1.2-1.4 is intermediate cohesiveness and  $\geq 1.4$  is

high cohesiveness. Solubility of flour can refer to the amount of powder that can dissolve in liquid.

**Table 10. Physical properties of the developed product**

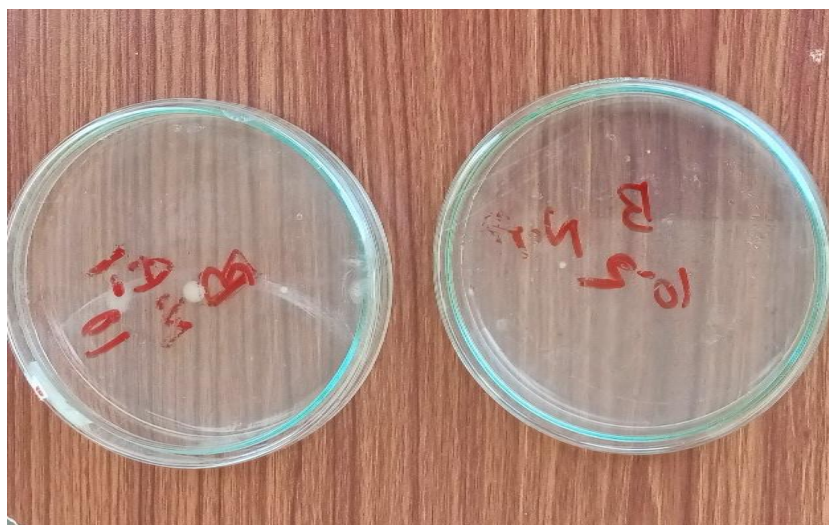
Sample	Bulk density (g/cm <sup>2</sup> )	Tapped density (g/cm <sup>2</sup> )	Hygroscopicity (%)	Flowability (Carr's index) (%)	Hausner ratio (HR)	Swelling index (%)	Water solubility index (WSI)
Protein mix	0.37±0.00	0.62±0.00	10.46±0.41	40.32±0.11	1.67±0.05	4.35±0.21	79.2±1.13

**3.6 Microbial analysis**

The developed Protein mix were analysed 0, 2 & 4 months of storage where total plate count, yeast and mold count were determined. The developed product showed no growth. The results were found to be good for the developed product which

are well supported by data on water activity of the developed product.

Overall, the developed product was found to be stable and acceptable at the end of 4<sup>th</sup> month study period.



**Fig. 7 The developed product shows no microbial growth**

## 4. CONCLUSION

The present work was undertaken where the product is combination of cereals, legumes and energy sources were used for the development of instant plant-based protein mix which is for the athletes as well as for the fitness adherents. It was observed that the developed product is exhibiting the higher levels of protein when compared with the commercial product. The product is also exhibiting the anti-inflammatory and anti-oxidant properties due to addition of totola flower. Addition of spices and nuts gave good flavor to the product.

The developed product is studied for the storage shelf life stability. The product was kept in aluminum pouches over a period of 4 months. The pH was changed from the 4.6 to 5.3. The color, titrable acidity, water activity and other physical properties of the product was studied. The sensory analysis of the product was done, the developed product is slightly higher in terms of appearance, color and texture and is highly acceptable when compared with the commercial product. Microbial analysis of the product was done; the product shows no growth. Overall, the developed product was found to be stable and acceptable at the end of 4<sup>th</sup> month study period.

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