

Formation of Vegan, Lactose-free Protein Powder Enriched with Vitamin D & study its Sensory Properties

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Abstract

*Malnutrition and deficiencies of nutrients in the common public is very much prevalent in India. One such vital nutrient is protein. 2 in 3 people or 73 percent population in India are hypoproteinemic, so protein should be added to the diet^[1]. In children with prolonged protein deficiencies, diseases like Kwashiorkor and Marasmus are known to develop^[2]. Pulses and legumes have been regarded as the poor man's meat when it comes to protein. Therefore, the product prepared focuses on deriving most of its protein content from pulses, legumes, and beans. A vegan protein supplement was made in powdered form, while keeping in mind a larger consumer spectrum, therefore making it vegetarian and lactose free, as major population in India is either vegetarian or lactose intolerant^[3,4]. Enrichment of Vitamin D was also done. The purpose was to create a product from commonly used items, keeping it low cost and away from the existing myths. The main ingredients used were Soybean (*Glycine max*), Amaranth (*Amaranthus*) seeds, Peas (*Pisum sativum*), Lentil dal (*Lens culinaris*), Agathi (*Sesbania grandiflora*) leaves, Groundnut (*Arachis hypogaea*) and Oyster Mushrooms (*Pleurotus ostreatus*). Methods used were germination, roasting, drying, parching, blanching, dehydration, and homogenization. Organoleptic properties were studied using triangle test, 9-point hedonic test and ranking test. While nutritional evaluation was done for energy, protein, carbohydrate, fibre, fat, ash, and vitamin D content. Different statistical analysis was done by calculating mean, standard deviation. It is also a good source of energy. Sample H3c, with a sensory score of 8.6 ± 0.48 was found to be the most acceptable by the panellists. Sample H3c was found to be a rich source of energy (333.44 Kcal), protein (29.73g), calcium (191.41mg), and vitamin D (321.55 µg). It can help against PEM and relieve the effects of nutritional deficiencies.*

Keywords Lactose-free; Protein; PEM; Supplement; Vegan.

1. Introduction

The powder was developed using different ingredients available at household level. Ingredients used are Soybean (*Glycine max*), Amaranth (*Amaranthus*) seeds, Peas (*Pisum sativum*), Lentil dal (*Lens culinaris*), Agathi (*Sesbania grandiflora*) leaves, Groundnut (*Arachis hypogaea*) and Mushrooms (*Agaricus bisporus*). Germination, blanching, parching, roasting, drying, dehydrating, grinding and homogenization were the methods used for the preparation of the product. The obtained powder is a rich source of energy, protein, carbohydrates, vitamin D and many minerals.

Legume or pulses belongs to the Fabaceae family, the cultivation of grain legumes is done for seeds, which in turn are used for consumption by humans or animals, as well as to produce oils for use in industries. ^[5] As far as the nutritional values are concerned, legumes are known to be important sources of protein, carbohydrates, dietary fibres, and minerals. ^[6] Legumes are also reportedly an excellent source of resistant starch, which gets broken down in the large intestine, producing short-chain fatty acids (like butyrate), which in turn are used by the cells of the intestine for food energy. ^[7] Studies on preliminary levels in humans are inclusive of potential for the consumption of legumes on a regular basis in plant-based diets, accounting for the reduction of prevalence of development of metabolic syndrome. ^[8] There is also evidence that suggests that dietary consumption of a cup of pulses every day helps in reduction of blood pressure as well as LDL cholesterol levels. ^[9]

When non grasses are used in the same way as cereals, the term pseudocereal is given to them, because all true cereals are grasses by nature. Majorly popular and widely used pseudocereal crops include amaranth and quinoa. Pseudocereals consist of a larger quantity of proteins as compared to actual cereals. Amaranth contains a considerable amount of lysine and tryptophan, which is a rarity in other cereals. Pseudocereals are particularly good sources of methionine, homocysteine, cysteine, and taurine, as compared to true cereals. High amount of essential fatty acids (EFAs) is also found in pseudocereals. Minerals like magnesium, iron and phosphorus are also found in rich quantities in pseudocereals. Folate content of amaranth and quinoa respectively is 4x and 10x that of true cereals. Significant amounts of phytochemicals are also found, like phenolic compounds, carrying antioxidant properties. ^[10] Mushrooms are a very potential source of macro as well as many micronutrients. It is rich in proteins, polysaccharides, unsaturated fatty acids, minerals, and vitamins, particularly vitamin B and vitamin D. minerals like magnesium, phosphorous, copper, selenium and potassium are found in abundance in mushrooms. It is also a great source of dietary fibre, β -glucan, and chitin. Apart from these mushrooms are also enriched with bioactive compounds which exhibit antioxidant properties, like flavonoids and phenolic compounds. ^[11] Mushrooms when exposed to sunlight or UV light generate significant amounts of nutritionally relevant vitamin D. From a study conducted on 30 healthy adults, it was found that vitamin D from mushrooms was as effective in increasing and maintaining serum 25(OH)D₂ levels as done by supplemental vitamin D. ^[12]

Peanuts, Goober (US), groundnuts, or monkey nuts (UK) are different names for the taxonomically named *Arachis hypogaea*; which is a leguminous crop grown for the seeds. Peanuts are a rich source of proteins, carbohydrates, lipids, vitamins, and minerals. It also contains purines and organic acids in appreciable amounts. Peanuts contain essential amino acids, essential lipids, and are a good source of energy and carbohydrates; and because of these aspects are a preferable food product to combat malnutrition. ^[13] Agathi or commonly named as hummingbird goes by the taxonomic name of *Sesbania grandiflora*. The leaves of agathi plant are a great source of sterols, vitamin C, kaempferol, myricetin, saponin and calcium. ^[14,15]

2. Materials and Methods-

Preparation of powder was done using soybean, amaranth, peas, lentil dal, agathi leaves, ground nut and oyster mushrooms. These raw ingredients were brought from local stores of

Gurugram, Haryana. Equipments used during the study were sprouter, wok, spatula, dehydrator, mixer, grinder, homogenizer, muslin clothes, microwave, gas burner, knife and 120 mesh BS sieve. All the materials were procured from the Nutrition Lab of Amity University Haryana.

2.1 Methods-

2.1.1 Germination-Germinating or sprouting is used interchangeably. Germinating is the process by which a sapling grows from a seed. With germination, improvement in nutritional and reduction in anti-nutritive compounds is generally seen. The process begins with hand picking the seeds for removal of stones or other impurities, followed by washing with water to remove dirt and remaining impurities. Clean seeds are then subjected to soaking for 30 minutes. Soaked seeds are placed in muslin cloth for 3 days or till germination, whichever is earlier. Seeds were washed and cleaned every 8 hours and were kept in cool and damp place. To produce flour, amaranth seeds were subjected to sprouting.

2.1.2 Dry Roasting-It is the method of cooking which uses dry heat. Clean pulses, cereals or seeds are dry roasted for various purposes. It reduces anti-nutritive factors and improves taste, aroma as well as nutritive value of food roasted. Roasting is done by using hot wok. Soybean, lentil dal and groundnuts were roasted separately on a low flame for 5-6 minutes. Change in colour is observed.

2.1.3 Parching-It is one of the methods of dry heat cooking. In this method very high temperature is used to dry different foods like pulses and cereals. In this method, pulse is first cleaned and is then soaked in water for 5 minutes. Soaked pulse is then partially dried in sun for 15 minutes. Then partially dried pulse is placed in a wok containing hot sand. Temperature is maintained at 190-200 degrees. Pulses are removed from sand after 80-90 seconds. Peas were parched using this method.

2.1.4 Blanching-In this method, the fruit or vegetable is first cleaned and then immersed in hot boiling water for 3-5 minutes, and then is transferred to cold water immediately. It helps in removing anti-nutritional factors. Agathi leaves and chopped oyster mushrooms were subjected to blanching.

2.1.5 Dehydration-It is the process of removing excess water from fruits, vegetables, pulses and grains. Blanched agathi leaves and germinated amaranth seeds were subjected to dehydration for 2-3 hours till 95% dehydration.

2.1.6 Dry Grinding-In this process, large particles are grounded into either into coarse or fine powder. Dehydrated agathi leaves, amaranth seeds, roasted soybean, lentil dal, peas, mushrooms, and groundnuts were subjected to dry grinding individually. Fine powder of all the ingredients was obtained.

2.1.7 Sieving-It is the process of passing the food or other ingredients to achieve an even particle size. 120 mesh BS sieve was used for sieving all the powders.

2.1.8 Homogenization-It is the process of mixing all the ingredients in way that it cannot be separated. All the obtained powders were taken in required quantities after sieving and mixed using a homogenizer.

2.2 Preparation of Mushroom powder-

Procured mushrooms were initially subjected to cleaning and fine chopping. Chopped mushrooms were then blanched in boiling water containing 2% salt and 0.01% citric acid for 3 minutes. Followed by placing in cold water for 5 minutes and were sieved. Mushrooms

were then sun dried for 2 days till 5% moisture level was left. Mushrooms were then grounded into a fine powder using grinder and were then passed through a sieve of size 120 mesh. Obtained powder was finally added to other ingredients for the process of homogenization.

2.3 Standardization of Recipe-

Recipe which is tried, tested, evaluated, and finally adapted by any food service organization is the standardized recipe. Its focus is consistency even after multiple batches. It gives consistent yield, consistent quality because of exact measurements, equipment, and procedure.

2.4 Steps involved in making the product-

1. Amaranth seeds are cleaned and kept for germination.
2. Soybean, lentil dal and groundnut were roasted separately. After roasting the ingredients were kept aside to cool.
3. Soybean and lentil dal were then ground into a fine powder.
4. Skin of groundnut was removed and ground into fine powder.
5. Peas were parched for 80-90 seconds and were kept aside to cool.
6. Agathi leaves were blanched for 3-5 minutes and were dried at normal conditions for 30 minutes.
7. Germinated amaranth seeds and agathi leaves were then placed in dehydrator for the process of dehydration.
8. Dehydrated amaranth seeds, agathi leaves and parched peas were ground separately into a fine powder.
9. All the formed powders were then passed through 120 mesh BS sieve. After sieving the ingredients were subjected to homogenization using the homogenizer.
10. Mushroom powder was then added to the homogenizer. Let the homogenizer mix all the ingredients for 3-4 minutes.

2.5 Sample preparation

Four different samples were prepared with the variation of amaranth seeds and groundnuts. Samples were named as 'H1a', 'H2b', 'H3c' and 'H4d'. The composition of soybean and peas were kept constant in all the four samples at 25g each. 10 g each of mushroom, agathi leaves and lentil dal flour was used in each sample. The different composition of amaranth seeds was 15g, 12.5g, 10g and 7.5g in sample 'H1a', 'H2b', 'H3c' and 'H4d' respectively. While, the composition of groundnut was kept at 5g, 7.5g, 10g and 12.5g in sample 'H1a', 'H2b', 'H3c' and 'H4d' respectively.

Ingredients (Powder)	H1a (g)	H2b (g)	H3c (g)	H4d (g)
Soybean	25	25	25	25
Peas	25	25	25	25
Amaranth seeds	15	12.5	10	7.5
Lentil dal	10	10	10	10
Agathi leaves	10	10	10	10
Groundnuts	5	7.5	10	12.5
Mushroom	10	10	10	10

Table 1: Representation of variation in all the four samples



Image 1: Flours of different ingredients 1- Soybean; 2- Peas; 3- Amaranth seeds; 4- Lentil dal; 5- Agathi leaves; 6- Groundnut; 7- Mushroom



Image 2: Sample H1a, H2b, H3c and H4d

3. Sensory Evaluation-

3.1 Triangle test-

Panellists for sensory evaluation were selected by triangle test. 30 people were selected for triangle test. They were served 3 cup coffee labelled as A, B and C. 2 contained same amount of sugar while 1 had less sugar. 20 were able to distinguish the odd sample and were selected for sensory evaluation.

3.2 9-point Hedonic test-

Selected 20 panellists were given 4 samples of powder mixed in water named as 'H1a', 'H2b', 'H3c' and 'H4d'. 20g of each sample was mixed in 200 ml water. The samples were then rated on different parameters like taste, texture, acceptability, and colour. Panellists rated them on a scale of 9, 9 being like extremely and 1 as dislike extremely. Scores of hedonic tests were used to give ranking to different samples. Mean of hedonic test were calculated for all samples and was then ranked as first and followed by other subsequent rankers.

4. Nutritional Analysis-

Nutritional analysis was done by AOAC method 2005. It was done to find nutritional properties of the product. Calories, carbohydrates, protein, fats, fibre, ash, and moisture per 100g were calculated for the effectiveness of the product. Vitamin D was also tested for availability in the product.

Estimation of carbohydrates was done using Anthrone method, proteins by Micro-Kjeldahl method and fats by Soxhlet apparatus method.

Energy- calculations were done by multiplying carbohydrates and proteins by four, and fats by nine and then adding up the total.

Moisture content- It was done using AOAC's oven method. 5 g of sample was weighed in clean and dried dish. Place the sample containing dish in the pre-heated oven (maintained at a temperature of 130°C) for 2 hours. Measure the weight at 30 minutes interval after cooling the sample. Weights until three constant readings are recorded.

Moisture content (%) = $(W_1 - W_2) * 100 / W_1 - W$

Where, W_1 = Weight of dish with the material before drying (g)

W_2 = Weight of dish with the material after drying (g)

W = Weight of empty dish (g)

Total Ash- 10g of sample was taken in silica crucible. Heat the crucible in muffle furnace for 4-5 hours at 600°C. Continue the ignition of the sample till grey ash is obtained. Cool the ash in desiccator and weigh. Repeat the process for half hour and take the reading. Repeat till three constant readings are obtained.

Ash (%) = $\text{Weight of ash} * 100 / \text{Weight of sample taken}$

Calculation of dietary fibre was done using Enzymatic-Gravimetric method. Calcium levels were calculated using flame atomic absorption spectrometry. Vitamin D was calculated using AOAC method.

6. Results-

9 scale Hedonic rating test was carried out by panellists selected from triangle test. The results were then analysed and interpreted.

6.1 Interpretation of sensory scores

Parameter	Sample H1a	Sample H2b	Sample H3c	Sample H4d
Taste	8.05±0.80	8.5±0.67	8.75±0.43	7.3±0.71
Texture	8.05±0.66	8.45±0.49	8.5±0.5	7.35±0.72
Colour	8.15±0.79	8.75±0.43	8.75±0.43	7.65±1.06
Acceptability	7.9±0.62	8.45±0.58	8.55±0.49	7.75±0.94
Overall	8.15±0.72 ^c	8.45±0.066 ^b	8.6±0.48 ^a	7.5±0.67 ^d

Table 2: Results of 9 scale Hedonic test (Mean ±Standard Deviation)

Sample H3c was the most accepted sample, followed by Sample H2b which is further followed by Sample H1a. Sample H4d is the last ranked sample among all samples.

6.2 Nutritional Analysis-

Sample H3c was subjected to nutritional analysis. It was done using AOAC method, 2005. Results were obtained for per 100g of the sample.

NUTRIENT	AMOUNT (per 100g)
Energy	1395.11 KJ (333.44 Kcal)
Carbohydrates	33.74 g
Proteins	29.73 g
Fats	8.48 g
Fiber	16.71 g
Moisture	7.27 % by mass
Ash	2.76 % by mass
Vitamin D	321.55 µg
Calcium	191.41 mg

Table 3: Results of nutritional analysis per 100g of sample

7. Conclusion

Starting out with an aim to develop something that helps in curbing protein deficiencies, especially in the poor sections of the society, this study has seen the development of a protein supplement from common everyday ingredients readily available to everyone. Generally regarded as the poor man's meat, pulses and legumes were naturally the first choices for deriving vegan protein for the supplement to be made. A vegan, lactose free and vitamin D enriched powder was kept in mind; while avoiding myths and keeping the cost low, so as to encourage the economically repressed sections of the society towards a healthier diet. The main ingredients used were Soybean (*Glycine max*), Amaranth (*Amaranthus*) seeds, Peas (*Pisum sativum*), Lentil dal (*Lens culinaris*), Agathi (*Sesbania grandiflora*) leaves, Groundnut (*Arachis hypogaea*) and Mushrooms (*Agaricusbisporus*). This ensured a good amount of energy, protein, Vitamin D and Carbohydrates in the resultant powder.

The powder developed was vegan as it is devoid of any kind of animal product. The product is also lactose-free, thus can be consumed by people with lactose intolerance. In the nine-point hedonic test, the best faired out sample turned out to be sample H3c, with an overall score of 8.6 ± 0.48 . This sample contained 10g of Amaranth seeds and 10g of Groundnuts. Once finalized as the most acceptable and liked sample. H3c was subjected to AOAC testing method. Wherein it gave promising results per 100g of sample. It contained 333.44 Kcal, 33.74g carbohydrates, 29.73 g protein, 8.48g fat, 191.41 mg of calcium and 321.55 µg of Vitamin D. With presence of good proportion of proteins and being rich in calories, the powder can be used a supplement for protein energy malnutrition. As the product is made from all commonly consumable foods, is free from lactose and is vegan, it can be consumed by all.

8. Word of Caution-

Supplementation should be done only after consultation with your doctor or nutritionist.

9. Declaration of Interest-

Authors declare no conflict of interest.

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