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RESEARCH PAPER

Design and development of an internet of things-based sprouting chamber for high-protein ready-to-serve supplements for adolescent girls

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Abstract

Adolescence is a crucial period for both men and women. The growth of the human body changes drastically during this time. The nutrition supply at the mentioned stage is of much importance. Germination is an outstanding green food development technique to increase the seed's nutritive profile in terms of quality. This study aimed to formulate protein-rich nutritional supplements for girls by utilizing an IoT-enabled sprouting chamber, ensuring enhanced precision, real-time monitoring, and improved quality control throughout the production process. A total of fifty variations of protein-rich powder were prepared, and their nutrient analysis was done using Design Expert Software. The R^2 value was found to be 0.98, and the P-value of (<0.0001) is considered significant. The sensory analysis was carried out by affective analysis by hedonic test, which relies on the product acceptance and preference levels. The nutritional analysis of this formulation had 18 g of protein, 2.5 g of fat, and 4% of fiber. The shelf life of the powder is found to be 12 months, and it can be stored at room temperature. Shelf life, cost-effectiveness, nutrient analysis, and the antimicrobial test showed a positive approach supporting the present research for sprouting grains' technological exploration.

1. Introduction

Nutrition is the science of foods, nutrients, and other substances, their action, interaction, and balance concerning health and disease. Nutrition and health are two sides of the coin and therefore inseparable. Malnutrition denotes impairment of health arising either from deficiency or excess, or imbalance of nutrients in the body (Reber *et al.,* 2019). Adolescence is the period of growth, reproductive maturation, and developmental transitions, demanding increased nutritional intake, making adolescents more vulnerable to dietary deficiencies (Arunjyothi and Jahan, 2021). Malnutrition was corrected using a health mix by providing appropriate nutrient content according to their daily needs. Sprouting is a simple and effective method for the improvement of the nutritional values of the seed. Germinating chambers have no negative environmental impact (Ortiz *et al.*, 2021). Cereals and protein-rich sprouted grains provide maximum nutrient content as raw materials. During germination or malting, many biochemical modifications occur, impacting product properties such as shape, bioactivity, consistency, flavor, and digestibility (Ikram *et al*., 2021)

The Internet of Things (IoT) is the network of physical devices, vehicles, home appliances, and other items embedded with electronics, software, sensors, actuators, and connectivity, enabling these objects to connect and exchange data. Node MCU is an open-source IoT platform. Sprouting of legumes greatly influences nutritional quality by increasing the bioavailability of nutrients and enhancing the digestibility and utilization of nutrients (Benincasa et al*.,* 2019). Millets are a gluten-free crop widely cultivated around the world, such as pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*) and sorghum (*Sorghum bicolour*) are selected as ingredients. They are highly nutritional when compared to major cereals such as wheat and rice (Mathangi, Geethanjali and Visalachi, 2017) and proved unique among grains due to calcium, dietary fibre, polyphenol proteins and antioxidant content (Prakash *et al.,* 2016). Micronutrients such as iron and calcium play an important role in mounting an immune response and substantially increase the risk of having poor immune responses to infections (Maggini *et al.,* 2018) in the adolescent age group. Healthy eating during adolescence is important as body changes affect an individual's nutritional and dietary needs.

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2. Materials and methods

2.1 Conventional and Internet of Things methods of sprouting

The raw material for the formulated protein-rich powder includes green gram, chickpeas, sorghum, pearl millet, finger millet, groundnut, almonds, palm jaggery, cardamom, red rice, and *Moringa* flower were collected from the retail shop in and around the Tiruchengode, Namakkal District. They were subjected to sprouting using the conventional method (white cloth) as in Figure 1a.

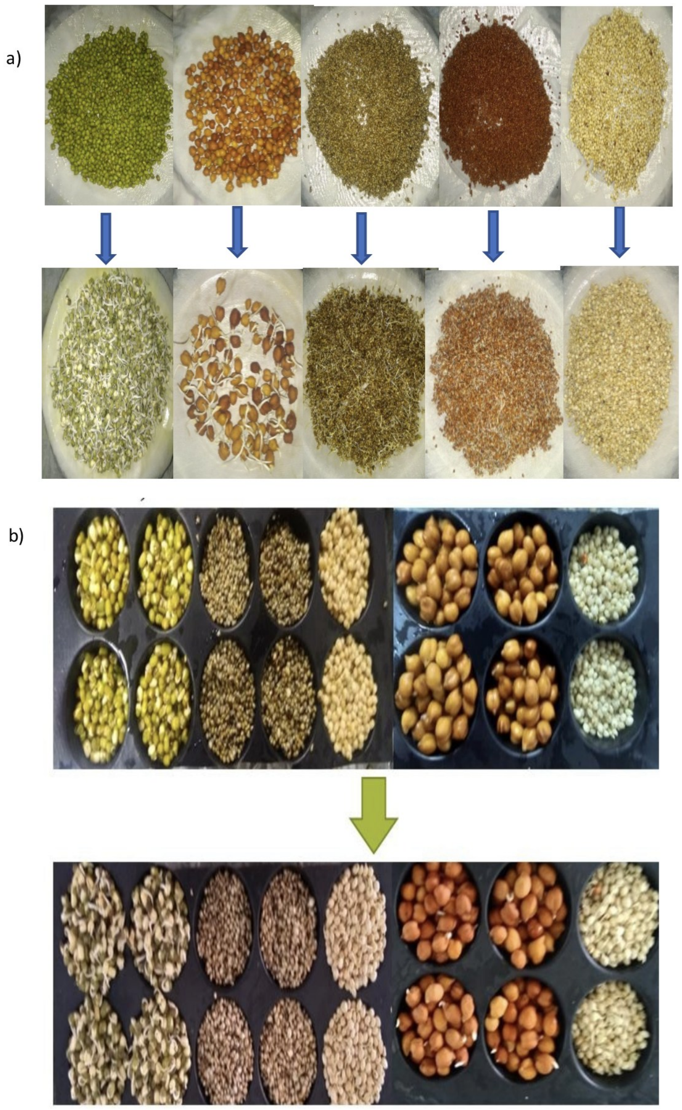

Figure 1. a) Conventional method of sprouts using white cloth and b) IoT based method of sprouts using IoT chamber.

The grains were washed twice with distilled water; after that, the grains were soaked for 8 to 12 h as a pretreatment. Then it was drained and tied using a white cloth and incubated for about three days using the conventional sprouting method. (Saleh *et al.,* 2013). They were subjected to sprouting using the IoT method (chamber) as in Figure 1b. The sprouted grains were collected and shade-dried for three days to remove moisture content. They were stored in an airtight container separately to prevent contamination and maintain stability.

2.2 Formulation of protein-rich powder

A total of 50 variations of protein-rich powder were formulated as per the design expert 7, as that of Installation > new file> response surface > central composite design > numeric factor. The sprouted grains are used for the Formulation of protein-rich powder. Protein content and overall acceptability were considered as the response for the Design expert. The sprouted grains were roasted to remove the moisture content, and the appropriate amount of raw material was taken and ground into fine powder.

2.3 Nutrient analysis tests

2.3.1 Protein

The protein estimation was done according to Lowry's method. Solution A, solution B, and C and Folin's phenol (1:1) were prepared. Bovine serum albumin was used as the standard. Stock solution and working standard were prepared in varying concentrations. Then the corresponding solution C were added and kept incubation for 10 min. It was then followed by the addition of Folin’s phenol and kept incubation for 30 min in the dark. The same procedure was done using the formulated sample, and the values were analyzed based on the standard used by plotting the graph. The blue colour was obtained, and it is measured by optical density at 660 nm (Maher *et al.,* 2017; Rubavathi *et al*., 2024).

2.3.2 Carbohydrate

Anthrone’s method was used to estimate the total carbohydrate content for the formulated protein-rich powder. Anthrones reagent (200 mg of anthrone was dissolved in 100 mL of 95% sulphuric acid) was prepared. Glucose is taken as a standard at different concentrations (Blank, 0.2, 0.4, 0.6, 0.8, 1.0 mL) of working standard, and 0.5 mL was taken as a test sample. Add 4 mL of anthrone reagent to each tube and incubate for 10 min in a water bath; after incubation, the green colour develops, and the absorbance was measured at 620 nm (Buckan, 2015).

2.3.3 Cholesterol

Zak’s method was used to estimate the total cholesterol content in the formulated protein-rich powder (Zaks, 1953) . The ferric chloride acetic acid reagent (0.05% ferric chloride in acetic acid) was used to extract the sample. Cholesterol was taken as a standard in different concentrations (Blank, 0.2, 0.4, 0.6, 0.8, 1.0 mL) of the working standard, and 0.5 mL was taken as a test sample. After adding standard and test samples, the

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ferric chloride acetic acid reagent was added and made up to 4 mL. To all 5 tubes, 3 mL of concentrated sulphuric acid was added and incubated for 20 min at room temperature. The absorbance of the reddish-pink colour was measured at 560 nm (Dinh *et al.,* 2011; Rubavathi *et al.*, 2020).

2.3.4 Crude fiber

The formulated protein-rich powder (2 g) was taken and treated with 150 mL of concentrated sulphuric acid, and the sample was treated with 150 mL of sodium hydroxide. Again, the sample is filtered. The sample was weighed and introduced into a muffle furnace at 550°C. The final ash content was weighed and noted (Reddy *et al.,* 2017; Chandaka *et al.,* 2017).

Crude fibre = [100×(Weight of treated sample) - (Weight of residue sample)]/ Weight of initial sample

2.3.5 Energy value

The National Institute of Nutrition calculated the approximate energy value for the formulated protein-rich powder in "NUTRITIVE VALUE OF INDIAN FOOD" by the National Institute of Nutrition. The energy was calculated and reported as kcal/100 g (Usharani *et al.,* 2013).

2.4 Product properties of formulated protein mix

2.4.1 Bulk density

The Narayana Rao method was used to determine the bulk density. Graduated cylinder tubes were weighed, and the formulated sample was filled to 5 mL by constant tapping until there were no further volume changes. The content was weighed, and the difference in weight was determined. The bulk density was reported as g/mL of sample (Khan *et al.,* 2016).

Bulk density = Weight of the sample taken/volume occupied by the sample

2.4.2 Moisture and ash content

In the present study, Total moisture content was analyzed by the Air-oven drying method, and ash content was determined by the Dry ash method (Usharani *et al.*, 2013; Azhar and Saini, 2016)

2.4.3 pH test

The protein-rich powder was mixed with distilled water, and the pH was measured by using litmus paper. Colour changes of the litmus paper were compared with the standard pH colour chart. The pH of this protein powder was found to be pH 6 (Momeni *et al.,* 2016).

2.4.4 Water solubility index

The formulated protein-rich powder (1 g) was taken and suspended in 20 mL of distilled water, and the sample was introduced in a water bath at 30°C for 30 mins, then the sample was centrifuged at 3000 rpm for 15 mins. The supernatants are collected in a separate dish for evaporation and stored at 120°C overnight (Sawant *et al.,* 2013).

$$WSI = \frac{Weight\ of\ solids\ in\ supernatant}{Weight\ of\ dry\ sample\ in\ the\ original\ sample} \times 100$$

2.5 Shelf life and microbial analysis of protein-rich powder

Microbial quality analysis of the protein-rich powder was performed following the Bacteriological Analytical Manual (BAM) procedures. A weighed amount of the sample was homogenized in sterile diluent, and serial dilutions were prepared for plating. Total viable bacteria were enumerated on Plate Count Agar after incubation at 35–37°C. Coliforms and *Escherichia coli* were detected using selective media such as Violet Red Bile Agar and confirmed on EMB or EC broth based on characteristic colony appearance. Yeasts and moulds were assessed by plating on acidified Potato Dextrose Agar and incubating at 25–28°C. Colony counts obtained from each test were expressed as CFU per gram of sample. The health mix was examined before and after every three months of storage (Usharani *et al.,* 2013; Farzana *et al*., 2017).

2.6 Sensory evaluation and cost analysis of formulated powder

The sensory evaluation of the formulated protein-rich mix was performed with the help of ten panel members. The sensory evaluation was done by organoleptic analysis through the parameters such as colour, flavor, taste, texture, and appearance. The rating for each characteristic was based on a nine-point scale (9-Like extremely, 8- Like very much, 7- Like moderately, 6 - Like slightly, 5- Neither like nor dislike, 4-Dislike slightly, 3-Dislike moderately, 2-Dislike very much, 1-Dislike extremely), Sulochana and Bakiyalakshmi (2011). The cost analyzed for the formulated protein mix was compared to the commercially available products (Rathnakumar and Pancharaja, 2018).

3. Results and discussion

3.1 Conventional and the Internet of Things method of sprouting

The grains which were selected, such as green gram, chickpeas, Pearl millet, finger millet and sorghum, were subjected to sprouting using the conventional and IoT method. The sprouting was fast in terms of IoT

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compared to the conventional sprouting method, with the optimum temperature and moisture content. Elongated sprouts were observed in the IoT-based method with high nutritive value.

3.2 Construction of the Internet of Things chamber

The automation of moisture and temperature levels in the chamber is maintained by the DHT11 sensor, which generates calibrated digital output. DHT11 can be interfaced with any microcontroller, like NodeMCU or Arduino, which introduces programming to novices. Controlled by NodeMCU, the data are transferred to the IoT server and retrieved through mobile applications. Sprouting of grains requires a temperature with the percentage of moisture level, which is to be maintained in a closed environment to sprout the grains in a better manner. So it is automatically done by specified hardware (NodeMCU, DHT11 sensor, DC fan) as represented in Figure 2. By using this automation system, a sprouting chamber was constructed for the optimum nutritive growth of the sprouts.

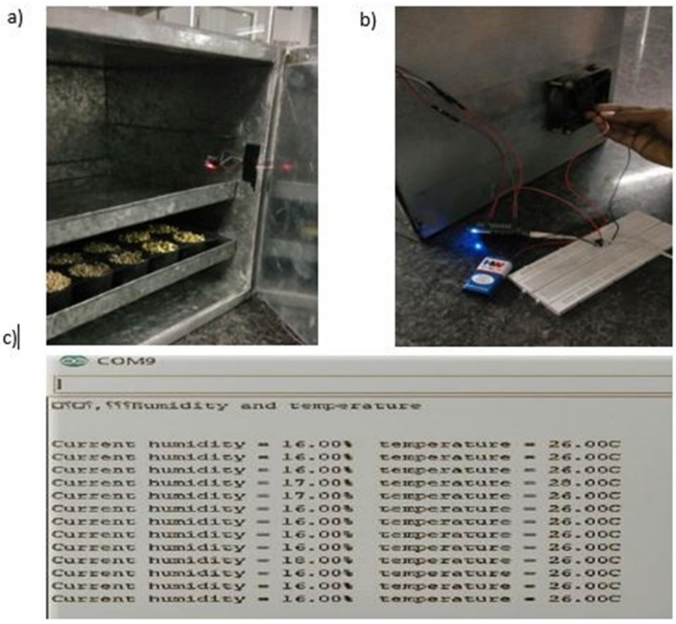

Figure 2. Working of IoT sprouting chamber, a) inside view of chamber, b) sensor working, c) output from the chamber.

3.3 Formulation of protein-rich powder

The Formulation of the sample was prepared by using Design-Expert software, where the five numeric factors were selected for the experiment, such as green gram, chickpeas, red rice, *Moringa oleifera* and groundnut. Some cereal grains, like rice, are consumed as whole grains; most cereals are converted to flour before usage (Morteza and Jamuna, 2016). Fifty different protein healthy mixes were formulated (Figure 3) with response to protein content and overall acceptability using Design Expert software. These variables show the different grains selected, and the formula of regression matches that of Reber *et al*. (2019). Their formulated mixture consisted of theirs was with 7 different samples with the same type of quadratic expression, which concludes that the formula is the same for the mixture of protein-rich supplements. The R^2 value was found to be 0.98, and a P-value of (<0.0001) was obtained, which indicated a better agreement between the actual and predicted values of nutritional analysis and confirmed a significant interpretation of the mathematical model.

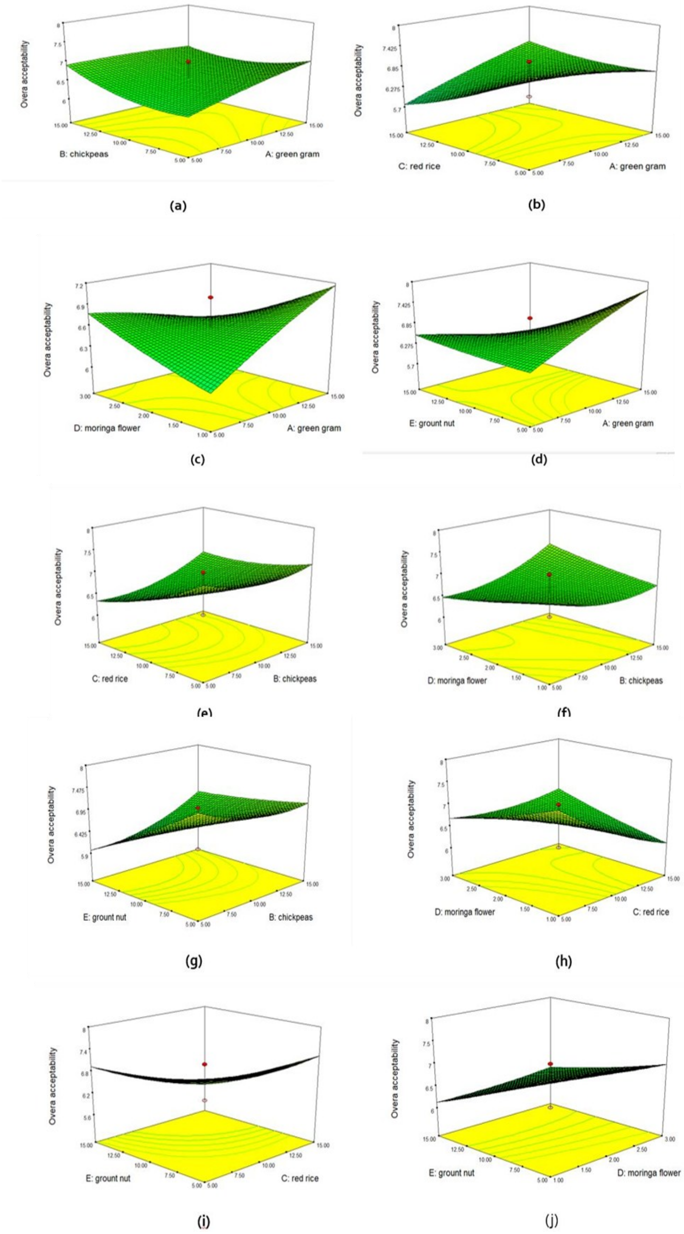

Figure 3. Effects of interaction between (a) green gram and chickpeas (b) green gram and red rice (c) green gram and *moringa* flower (d) green gram and groundnut (e) red rice and *moringa* flower (f) chickpeas and groundnut (g) *moringa* flower and groundnut (h) red rice and groundnut i) red rice and groundnut (j) *moringa* flower and groundnut.

Quadratic Regression equation of response with selected variables:

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Response	Quadratic Regression equation with 5
Y1	+26.62464+0.21898*X1-1.61415*X2-0.4397*X3-3.45533*X4+0.77643*X5-0.020862*X1*X2+0.10751*X1*X3-0.090688*X1*X4-0.029221*X1*X5+0.024663*X2*X3+0.43406*X2*X4+0.033287*X2*X5+0.23969*X3*X4-0.054638*X3*X5+0.062563*X4*X5-0.017260*$X1^2$+0.020924* $X2^2$-0.046958* $X3^2$-1.08557* $X4^2$-0.023659 $X5^2$
Y2	+10.40753+0.34054*X1-0.38257*X2-0.44672*X3-0.96928*X4+0.044535*X5-7.5000E.033*X1*X2+0.017500*X1*X3-0.075000*X1*X4+0.02250*X1*X5+7.50000E-003*X2*X3+0.050* X2*X4+0.01250*X2* X5+.075000*X3* X4-0.012500*X3* X5+0.02500* X4* X5-1.80450E-003* $X1^2$+8.80210E.003* $X2^2$+5.26656E.003* $X3^2$+0.043276* $X4^2$+73103E.003* $X5^2$

Where, X1= Green gram; X2= Chickpeas; X3= Red rice; X4= *Moringa sp.,* flower; X5=Ground nut; Y1= Protein content in g/100g; Y2= Overall acceptability.

3.4 Comparative studies of protein content in grains

The protein content was analyzed for the grains under various conditions, such as grains without sprouting, grains sprouted using the conventional way, and the grains sprouted using the IOT chamber as a comparative study. The highest protein content was observed in all five types of sprouted grains using the IoT chamber, as illustrated in Figure 4. The protein content of the sprouted barley was 38.6% higher ($P < 0.01$) compared to that of raw grain (Ortiz *et al.*, 2021)

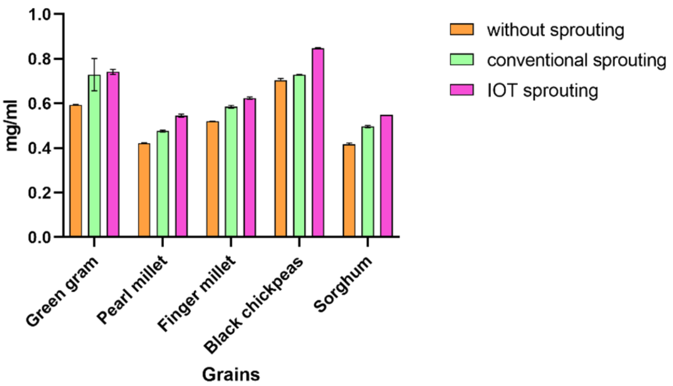

Figure 4. Comparative studies of protein content in grains.

3.5 Product properties of formulated protein mix

The product properties were analyzed for the formulated protein-rich powder. Formulation I was considered to be a normal mix, which was not subjected to sprouts. Formulation II was supposed to be a protein-rich mix which was subjected to sprouting using the conventional method, and Formulation III was considered to be a protein-rich mix which was subjected to sprouting using the IOT chamber. Our formulation was found to contain a moisture content ranging from 3.04 to 3.96%. The highest moisture content was found in formula 1 (3.96). The ash level of the formulated three samples ranged from 3.23 to 7.4%. The pH of the formulated protein-rich powder was found to be 6 ± 0.5. The highest water solubility index was observed in the sprouted grains by using the IoT chamber, which is the same as that observed in Mathangi and Geethanjali (2016). When compared with other commercial health mix powders, it allows better mixing with a solvent like water and milk. The properties like moisture, crude fat, fibre, carbohydrate content and energy values of *Laddu* (3.60%, 8.55%, 3.88%, 61.85% and 399 Kcal, respectively) were reported as in Shilpa and Pushpa (2014). The moisture content of 8.9%, carbohydrate content of 63.32%, protein content of 23.18%, and fibre content of 8.50 g were reported in Mathangi and Geethanjali (2016).

3.6 Nutrient analysis for protein-rich powder

The nutritional analysis was done for the formulated protein-rich mix, and comparative studies were made with a commercially available health mix. Based on our study, Formulation III have the highest nutrient value among the other two formulations. It has 18 g of protein, 2.5 g of fat and 4% of fibre as described in Figure 5. The nutritional value of grains will increase during sprouting. For maximizing the nutritive value of grains, we recommended the process of sprouting. The biochemical changes occurring during the sprouting process. Similarly, this result is in accordance with the observations reported by Elien *et al.* (2019). The protein content of sprouted barley was as high as 38.6% compared to that of raw grain, and it was reported by other researchers for cereal grains, soybean and pea seed as in Ortiz *et al.* (2021).

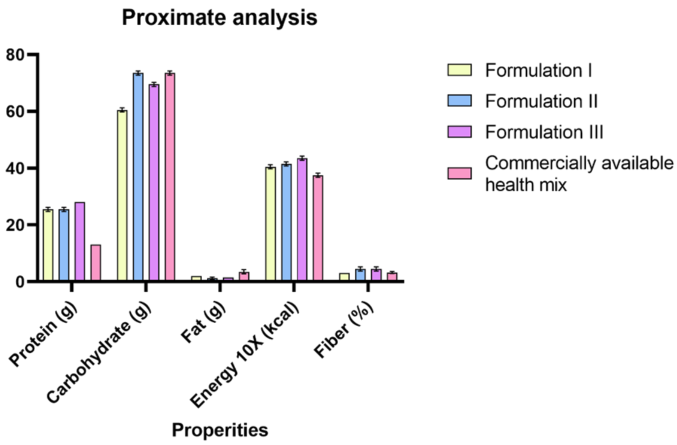

Figure 5. The nutritional content of the formulated protein mix per 100 g.

3.7 Shelf life and microbial analysis test

Microbial analyses such as total viable count, coliforms and *E. coli*, yeast and molds of protein-rich powder were carried out according to the procedure of the Bacteriological Analytical Manual. In this study, the total microbial count was assessed, and up to 12 months, the total mould and yeast count were within the acceptable limits. There is no *E. coli* or Coliforms found during the entire shelf-life period, as illustrated in Table 1. From this analysis, it was found to be safe for consumption up to a one-year period. After one year, the total microbial count will gradually increase. After the 6-month time period, the hygienic indicator organisms were found to be gradually increased, and the product quality became worsening was reported (Farzana *et al.*, 2017). During this analysis, there was no microbial growth in the initial stage and also one month period later was observed with the addition of no special preservatives. Mathangi, Geethanjali and Visalachi (2017).

3.8 Cost analysis for the formulated protein-rich mix

The cost of the formulated mix was analyzed with famous commercially marketed products. The prepared product cost was found to be Rs 130. On the basis of the sensory acceptability, it was found that Formulation 3 was scored high in terms of colour and appearance, texture, flavour, taste and overall acceptability. The rate for commercial products was Rs 180. So formulated product is affordable to all classes of people (Table 2). Compared to the commercial product, the cost of the formulated protein mix was low. The cost of the protein-rich flavoured bar per 100 g of dry ingredients at the prevailing cost of the raw materials was highest in T1 (Rs 29.33), followed by T2 (Rs 20.69), and T3 (Rs 20.34) was reported in Mukherjee (2021).

Table 2. Cost analysis for the formulated protein-rich mix.

Ingredients	Total quantity used (g)	Cost for total quantity (Rs)
Green gram	50	10
Sorghum	100	8
Chickpeas	100	16
Finger millet	100	5
Pearl millet	100	6
Groundnut	25	12
Red rice	25	7
Almonds	25	7
palm jaggery	25	10
Cardamom	50	5
Moringa flower	25	5
Total cost for a formulated mix		Rs 130 /-
Cost for commercial product		Rs 180 /-

4. Conclusion

Nowadays, consumers prefer food items with high bioavailability, outstanding sensory characteristics, and extended shelf life. The present research was carried out to study the problems in adolescent girls and to improve their health through the formulation of a protein-rich mix. The protein and nutrient analysis for the IoT chamber sprouted seeds shows a higher value compared to the conventional sprouting techniques. A formulated protein-rich mix was evaluated for sensory evaluation. The formulated protein-rich powder had a high nutrient content and energy values compared to the commercial health mix. This study concluded that the formulated protein-rich mix would have a potential effect to improve the health of an individual. As the powder is rich in protein, iron, and other macronutrients, it can be recommended for children, athletes, and the person who are suffering from malnutrition and anemia due to its lower cost and various beneficial effects with fewer side effects. Moreover, this study follows a simple methodology and eco-friendly techniques. The cost of a commercial product is Rs 180, which is affordable for all people.

Conflict of interest

The authors declare that they have no conflict of interest regarding this investigation.

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Table 1. Microbial analysis of formulated protein powder.

Test parameter	Shelf life (Months)						
	0	3	6	9	12	15	18
Total viable count, CFU/g	A	A	A	1.9×10^2	9.0×10^3	3.6×10^6	N
Total yeast and moulds Count, CFU/g	A	A	A	A	A	5.0×10^2	5.0×10^2
Total coliforms, MPN/g	A	A	A	A	A	A	A
E. coli, MPN/g	A	A	A	A	A	A	A

A: Absence, N: Numerous.

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