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Research Article

Prebiotic Nutraceutical for Geriatrics

Development of Functional Prebiotic Nutraceutical Supplement Mix for Geriatrics

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Abstract: This study aims to develop a functional prebiotic nutraceutical supplement mix for geriatrics and evaluate its nutritional and sensory characteristics. Prebiotics are non-digestible food nutrients that selectively stimulate the activities of beneficial probiotic organisms in the colon and maintain intestinal microbial balance. Nutraceutical includes functional foods, dietary supplements, and medical foods. Geriatrics is a medical practice that addresses older patients' complex needs and maintains functional independence even in the presence of chronic diseases. Elderly people are at risk for macro and micronutrient deficiencies due to various social, physical, and economic obstacles. This research product aims to promote the ideal blend of grains, legumes, and nuts for elderly people and enhance their nutritional energy. Foxtail millets, oats, garlic, almonds & cashew nuts possess high prebiotic content. This study formulated ten formulations using Response Surface Method – Design Expert 12.0. The third sample (Trial 3) containing foxtail millet (25%), green pea protein isolate (10%), oats (15%) and carrot (15%), pomegranate (15%), garlic (5%), almond & cashew nut (5%), skim milk solids (10%) had the highest score for sensory attributes. The developed combination was high in nutrients and provide ample protein and energy for daily needs. Its nutritional value for 100g is of total protein (11.5%), fat (4.36), carbohydrate (67.57), vitamins, minerals, and total energy (360.17 K/Cal).

Keywords: Geriatrics, Health mix, Nutraceutical, Prebiotic, Response Surface Method.

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1. INTRODUCTION

Geriatrics is a practice that focuses on the advanced wants of older patients. Geriatric medicines aim to improve older adults' health by preventing and treating diseases and disabilities. Aging may be an advanced and ineluctable organic process related to several persistent weakening health effects. The leading causes of all deaths worldwide are infectious diseases, lower metabolic process infections, diarrheic diseases, and ischemic heart diseases. People need fewer calories but more nutrients to maintain proper health as they grow older. To fill these nutrients space, fortified foods or beverage products will significantly increase healthy aging in consumers.[1] Prebiotics are a group of nutrients of fructooligosaccharides (FOS), galacto-oligosaccharides (GOS) that feed the intestinal microbes, and their nutrients are released into the blood circulation and other distant organs. The gut microbiota affects the functions of the intestines, like the integrity and intensity of the intestine. Prebiotics has a big impact on human health, creating them appealing agents for improving human life quality in the fight against cancer, tube sickness, obesity, and mental disorders. There are various studies on the helpful edges of prebiotics on human health; nonetheless, well-designed long-run clinical trials and genetic science analysis are needed to corroborate the health claims. The health mix has all the essential nutrient combos in one meal. This health mix contains a proper mix of grains, legumes, and nuts that are ideal for today's health-conscious consumers. This superb supply of high-quality macromolecules and vitamins will prevent numerous deficiency diseases. Macromolecule energy undernutrition could be a severe organic process deficiency that often happens throughout the necessary transition amount of a health geriatric mix, limiting an elderly's physical and mental development. This example is presented by introducing healthy blends in quality and amount with the right proportions.[2,3] Malnutrition-related protein metabolism and micronutrient insufficient have been strongly associated with immune function deterioration, which is already affecting people as they age. It emerges as a lack of cell-mediated immunity in elderly malnourished people. Low immunity raises the risk of infection and delays illness recovery. Malnutrition impairs wound healing and tissue repair, which can be ascribed in part to vitamin insufficiency. Malnutrition is a significant contributor to the onset of various geriatric disorders. Geriatric syndromes are complex disorders that affect people as they age and have significant health implications. It predisposes older people to wound-healing abnormalities and chronic wounds, which are a significant burden on patients and are linked to a lower quality of life. Geriatric syndromes are multifactorial illnesses affecting people as they age and have major health consequences. Sensory impairment, such as reduced taste or olfactory malfunction, delayed stomach emptying, and disrupted motility, contribute to aging gut function.[4,5,6] Recommended Dietary Allowance (RDA) is the amount of essential nutrient intake that the Food and Nutrition Board considers sufficient to meet the known dietary needs of almost all healthy individuals, as shown in Table 1.[7]

Table 1: RDA summary from the National Institute of Nutrition (NIN) and Indian Council of Medical Research (ICMR), Department of Health Research, and Ministry of Health and Family Welfare of Government of India [NIN & ICMR 2020].

Nutrition parameters	MEN (65kg)	WOMEN (55kg)
Energy (Kcal/day)	2110	1660
Carbohydrate (g/day)	130	130
Protein (g/day)	54	45.7
Fats (g/day)	25	20
Vitamin B1 (mg/day)	1.4	1.4
Vitamin B2 (mg/day)	2	1.4
Vitamin C (mg/day)	80	65
Vitamin A (µg/day)	1000	840
Vitamin B12 (µg/day)	2.5	2.5
Calcium (mg/day)	1000	1000
Magnesium (mg/day)	385	325

Foxtail millet has 12.3% of protein content and 3.3% of minerals. Foxtail millets are rich in vitamin B, especially niacin B3 and thiamine B1. Oats are a good source of iron, magnesium, and zinc. Green pea protein powder is rich in amino acids, especially isoleucine, and leucine.[8,9]

2. MATERIALS AND METHODS

2.1. Materials

The raw materials, viz. foxtail millet powder, oats, and green pea protein powder, were procured from the organic supermarket, Erode. In addition, fresh carrots, pomegranates, garlic, dried almonds, and cashew nuts were obtained from the local organic supermarket, Erode.

2.2. Preparation of Carrot Powder

Carrots were blanched and sliced to 5-6mm thickness with a steel cutter and immediately placed in the dryer. The carrot slices were dried in a hot air dryer with an air velocity of 1m/s at 60°C for 5-6 hours. The dried sample was grinded and passed through a 60 mesh sieve, and the powder obtained was packed in an air-tight container.[10,11,12]

2.3. Preparation of Pomegranate Powder

Pomegranates were washed in tap water and drained. They were manually cut up, and the outer leathery skin was separated from the arils. These arils were dried in a hot air dryer with an air velocity of 1m/s at 50-60°C for 4-5 hours. The dried sample was grounded with an electrical kitchen grinder to powder and passed through a 60-mesh sieve. The powder obtained was packed in an air-tight conditioner.[13,14]

2.4. *Preparation of Garlic Powder*

Garlic bulb was sorted and peeled, and damaged ones were discarded. The sorted cloves were sliced into 2mm thick strips and immediately placed inside the hot air dryer and 40-50°C for 4.5-5.5 hours with air at a velocity of 1 m/s. The dried sample was grounded with an electrical kitchen grinder to powder form and passed through a 60-mesh sieve. The powder was packed in an air-tight conditioner.[15,16]

2.5. *Preparation of Almond and Cashew Nut Powder*

Cashew nuts and almonds were grounded separately with an electrical kitchen grinder into powdery form and passed through a mesh sieve. The powder was packed and sealed separately.[17,18]

2.6. *Preparation of supplement mix*

After preparing the ingredients, all those ingredients were added and mixed well, as mentioned in Figure 1. Finally, the supplement mix was stored at room temperature for further studies.

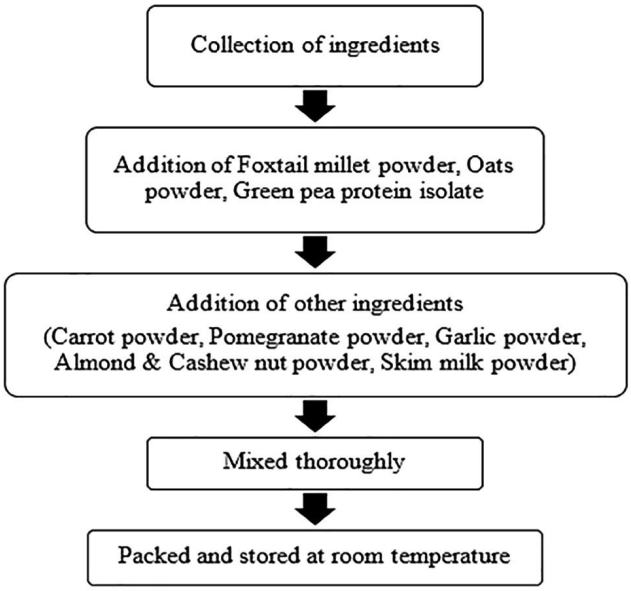

Fig 1: Flow chart for preparation of supplement mix

2.7. *Formulation of supplement mix*

The preliminary trials were conducted with all the raw materials in different proportions, as shown in Table 2. From these trials, firstly, the constant ratio of carrot, pomegranate, garlic, skim milk[19], almond, and Cashew nut powders were concluded, as shown in Table 3. Secondly, the ratio sets of the main ingredients, such as foxtail millet (20-25%), oats (15-20%), and green pea protein isolate (10-15%) powders, were determined. Finally, to obtain a constant percentage, Response Surface Method (RSM) was used, using ten different run factors, as shown in Table 4.

Table 2: Preliminary trials were conducted with raw materials in different proportions, (below mentioned values are in grams).

Ingredients	Trial i	Trial ii	Trial iii
Foxtail Millet Powder	-	30	25
Oats Powder	30	-	20
Green pea protein isolate	30	25	15
Carrot powder	10	10	10
Pomegranate powder	10	15	15
Garlic powder	15	10	5
Almond & Cashew nut powder	5	5	5
Skim milk powder	-	5	5

Table 3: After conducting preliminary trials in different proportions, we conclude with a constant the ratio for some ingredients (below mentioned values are in grams)

Ingredients	Weight (in grams)
Carrot powder	15
Pomegranate powder	15
Garlic powder	5
Almond & Cashew nut powder	5
Skim milk powder	10

Table 4: Using Response Surface Method (RSM) - Design expert 12.0. Other non-constant the ratio for some ingredients (below mentioned values are in grams)

Ingredients	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9	Trial 10
Foxtail millet powder	22.50	20.50	25.0	22.5	24.70	20.0	20.0	25.0	20.83	20.0
Oats powder	17.50	17.5	15.0	16.29	17.5	20.0	20.0	15.50	15.83	15.0
Green pea protein isolate	12.50	12.0	10.0	12.5	12.5	10.0	10.0	15.0	13.33	10.0

2.8. *Determination of Moisture content*

Moisture content was estimated by oven drying method[20]. First, an empty petri dish was weighed. Then, 5 g of the sample was placed in the weighed petri dish and weighed. Next, the oven was preheated to 100°C, and the dish was placed in the oven at 105 ± 2°C for 4 hours. Finally, the dish was removed, cooled in the desiccator, and weighed.

$$\text{Moisture, percent by mass} = \frac{100\,(M_1 - M_2)}{M_1 - M}$$

where,
M_1 =- mass, in g, of the dish with the material before drying,
M_2 =- mass, in g, of the dish with the material after drying to constant mass and
M = mass, in g, of the empty dish.

2.9. *Determination of Ash content*

Ash content was estimated by muffle furnace[20]. Empty crucible (M) was weighed, and 5 g of sample was placed in the crucible, weighed, and ignited for one hour in a muffle furnace at 500 ± 10°C for 4 hours until grey ash was obtained. The crucible was cooled in a desiccator and weighed.

$$\text{Total ash (on dry basis), percent by mass} = \frac{10\,000(M_2 - M)}{(M_1 - M)(100 - W)}$$

where,
M_2 = mass, in g, of the dish with the ash,
M_1 = mass, in g, of the empty dish,
M = mass, in g, of the dish with the material taken for the test and
W = percent moisture in the sample.

2.10. *Determination of Crude fiber content*

The crude fiber was determined[20]. After determining the total solid content and extracting for about one hour with petroleum ether, using a Soxhlet apparatus, 2.5 g of the sample was weighed. The fat-free material was transferred to a one-liter flask. 200 ml of dilute sulphuric acid was boiled and transferred the boiling acid to the flask containing the fat-free material and immediately connected the flask with a water-cooled reflux condenser and heated. The flask was rotated continuously, boiled for 30 minutes, filtered through fine linen, and washed with boiling water until the washings were no longer acidic to litmus. The residue on the linen was washed with 200 ml of boiling sodium hydroxide solution. The flask was connected to the reflux condenser and boiled for 30 minutes, and the flask was removed and filtered through the filtering cloth. The residue was washed with boiling water and transferred to a Gooch crucible. The residue was washed with hot water and with 15 ml of ethyl alcohol (95 percent by volume). The Gooch crucible and contents dried at 105 ± 2°C in an air oven and cooled and weighed. The contents in the crucible were incinerated in an electric muffle furnace at 600 ± 20°C until all the carbonaceous matter was burnt. The crucible was cooled and weighed.

$$\text{Crude fibre (on dry basis), percent by mass} = \frac{10\,000\,(M_1 - M_2)}{M_1(100 - W)}$$

where,
M_1 = mass, in g, of Gooch crucible and contents before ashing,
M_2 = mass, in g. of Gooch crucible containing ash,
M = mass, in g, of the material taken for the test and
W = percent of moisture content.

2.11. Determination of Protein content

The Kjedahl method[21] estimated protein content. 0.7 g to 2.2 g weighed sample, 0.7 g mercury oxide, 25 ml sulphuric acid, and 15 g powdered potassium sulfate were added to digestion. The flask was placed in an inclined position on a heater and heated until foaming ceased. Boiled until the solution becomes clear and then continued for 1 to 2 hours and cooled. 200 ml of distilled water and 25 ml of the sulphide or thiosulphate solution were mixed to precipitate mercury. A few zinc granules and 25 g of sodium hydroxide as a solid were added to make the solution strongly alkaline and immediately connected to the distillation bulb; the tip of the condenser was immersed in a measured standard acid in the receiver. Rotate the flask and heat until 150 ml of ammonia gets distilled. The receiver was lowered before stopping distillation, and the tip of the condenser was washed with distilled water. Excess acid was titrated with standard 0.1N sodium hydroxide; methyl red was used as an indicator.

$$\text{Nitrogen content (N) in g} = (A\text{-}B) - (C\text{-}D) \times 0.0014$$

where,
A = volume in ml 0.1N acid measured for main distillation,
B = volume in ml 0.1N alkali used for back titrating A,
C = volume in ml 0.1N acid measured for blank distillation and
D = volume in ml 0.1N alkali used for back-titrating C.

$$\text{Protein, percent by mass} = \frac{N \times 100 \times convention\ factor}{W}$$

where,
N = mass of nitrogen content in g of the original sample,
W= mass of sample in g.

2.12. Determination of fat content

The Soxhlet method20 determined the fat content. First, in a thimble, 10 to 30 grams of the sample was weighed and dried for 2 hours at 100 ± 2°C. Next, the thimble was placed in the Soxhlet extraction apparatus and extracted for 16 hours. Finally, the extract was dried, cooled in a desiccator, and weighed.

$$\text{Fat percent by mass} = \frac{100\,(M_1 - M_2)}{M}$$

where,
M_1 = mass, in g, of the Soxhlet flask with the extracted fat,
M_2 = mass, in g, of the empty Soxhlet flask clean and dry,
M = mass, in g, of material taken for the test.

2.13. *Determination of Carbohydrate content*

Carbohydrate content was estimated by early reported method [22]. Total carbohydrate is calculated after determining the percentage of moisture, protein, fat, and ash. Total carbohydrates, including sucrose, dextrose, and dextrin maltose or lactose, percent by mass,

= 100 - (A + B + C + D)

where,
A = percent by mass of moisture,
B = percent by mass of total protein,
C = percent by mass of fat and
D = total ash, percent by mass.

2.14. *Determination of Energy*

The Atwater general factor system calculated the energy,

$$\text{Energy (Calories/100g)} = \frac{(Fat \times 37) + (Protein \times 17) + (Carbohydrate \times 17)}{4.18}$$

2.15. *Determination of Vitamin B1*

Vitamin B_1 content was estimated by the chemical method as mentioned in Indian Standards 5398 (1969). [23]

$$\mu g \text{ of thiamine per } g = \frac{Test\ Reading - Blank\ Reading}{Recovery\ Reading - Test\ Reading} \times \frac{Dilution\ Factor}{Weigh\ of\ substance\ taken}$$

2.16. Determination of Vitamin B2

Vitamin B_2 content was estimated by chemical method. First, the sample solution was prepared (Section 4.3.1) [24]. Next, two cuvettes with 10 ml of sample solution were taken. Then, one milli-liter of the standard riboflavin solution and one milli-liter of water were added to each cuvette. Next, one milli-liter of acetic acid was added to both cuvettes and mixed well. While mixing, 0.5 ml of potassium permanganate solution was added and kept still for two minutes. Then, by mixing the solutions in both cuvettes, 0.5 ml of the hydrogen peroxide solution was added, which changed the permanganate color within 10 seconds. Next, the cuvettes were shaken vigorously to turn out the oxygen. After that, the cuvettes were tipped at the side to remove the gas bubbles on the top.
The calculation below was based on the values of the fluorometer,

$$\text{mg of riboflavin} = \frac{B-C}{A-B} \times \frac{1}{10} \times \frac{1}{1000}$$

where,
A = fluorescence of the sample solution containing the added one milliliter of the standard riboflavin solution,
B = fluorescence of the sample solution containing one milliliter of added water and
C = fluorescence of the sample solution containing one milliliter of added water and 20 mg of powdered sodium hydrosulfite.

2.17. Determination of Vitamin C

Vitamin C content was estimated by 2,6-dichlorophenol indophenol method (DCPIP)[25]. 5 g of sample was weighed and added with metaphosphoric acid. The volume of the solution was made to 100ml using the acid. After mixing the solution vigorously, it was filtered immediately using filter paper. Then, 10 ml of the filtrate was titrated with indophenol solution until the pale-pink color resisted for five seconds.

$$\text{mg of Vitamin C per 100g of sample} = \frac{A \times B \times 1000}{W}$$

where,
A = volume in ml of the indophenol solution used for titration,
B = weight in mg of the ascorbic acid equivalent to one milliliter of the indophenol solution and
W = weight in g of the sample taken for the test.

2.18. Determination of Vitamin A

The spectrophotometric Method estimated Vitamin A content as mentioned in Indian Standards 5886 (1970). [26]

1) To determine the corrected absorbance at 325 mμ,

$$E_{325}\,(\text{corrected}) = 6.815E_{325} - 2.555E_{310} - 4.260E_{334}$$

2) To determine the Vitamin A content in I.U. per 100 g of sample,

$$= \frac{E325\,(corrected) \times 1830 \times 100}{L \times C}$$

where,
L = length of the light path in absorption cell in cm and
C = amount of assay sample, in g per 100 ml, of isopropyl alcohol solution.

2.19. Determination of Vitamin B12

Vitamin B12 content was estimated by the microbiological method as mentioned in Indian Standards 7529 (1975). [27]

2.20. Determination of Calcium

Calcium content was determined volumetrically using ethylenediamine tetraacetic acid (EDTA)[28]. First, the sample was mixed with 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution, and 2 ml of potassium cyanide solution. Then, diluted with 150 ml of water and, using a pH meter, the solution was adjusted to 12.5, adding 20% sodium hydroxide solution. Next, 0.1 g of Patton and Reeder's indicator was added and stirred well, and titrated with 0.01M EDTA solution from a burette until the color changed from red to pure blue. Further drop-wise addition gave the pure blue end-point.

$$\text{Calcium (Ca), percent by mass} = \frac{V_1 \times 0.04008}{M}$$

where,
V_1 = volume in ml of EDTA solution consumed in titration and
M = mass in g of the sample in the solution taken for the test.

2.21. *Determination of Magnesium*

Magnesium content was determined volumetrically using ethylenediamine tetraacetic acid (EDTA)[28]. First, the sample was mixed with 10 ml of hydroxylamine hydrochloride solution, 2 ml of potassium cyanide solution, and 25 ml of triethanolamine solution. Then, the solution was diluted with 150 ml of water and, using a pH meter, was adjusted to 9.5, adding buffer solution. Next, four drops of Eriochrome Black T indicator were added, stirred well, and titrated with 0.01M EDTA solution from a burette until the color changed from red to pure blue. Further drop-wise addition gave the pure blue end-point.

$$\text{Magnesium (Mg), percent by mass} = \frac{0.0243 \times (V_2 - V_1)}{M}$$

where,
V_2 = volume in ml of EDTA solution consumed in titration,
V_1 = volume in ml of EDTA consumed in titration for calcium determination in the same aliquot of solution of sample and
M = mass in g of the sample in the solution taken for the test.

2.22. *Sensory analysis*

Twenty older adults did the sensory evaluation, and the highly rated trial was considered the final product. The tests were conducted according to the 9-point hedonic scale in Table 5. The main attributes considered in this evaluation were color, flavor, taste, consistency, and overall acceptability. [29]

Table 5: Sensory characteristics scale

9-point hedonic scale	Acceptability/rating scales
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	I neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

2.23. *Shelf life*

The storage test was conducted on two different days, on day 1 (D_1) and day 30 (D_{30}). The product was stored in an air-tight container. Sensory evaluation was carried out on the mentioned days. The evaluation was carried out as mentioned in Section 2.15. [30,31]

2.24. *Sample formulation*

For estimating specific responses, a quadratic polynomial regression analysis was used,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon$$

where, Y= response; X_1 = Foxtail millet; X_2 = Oat's powder; X_3 = Green pea protein powder; β_0 = interception; β_1, β_2, β_3 = linear terms; β_{12}, β_{13}, β_{23} = linear interaction term; β_{11}, β_{22}, β_{33} = quadratic regression co-efficient terms; ε = error.[32]

2.25. *Statistical Analysis*

The Central Composite design under Response Surface Methodology by Design Expert 12.0 software (Stat-Ease Inc., Minneapolis, United States of America) was used to run various factors and statistical analyses.

3. RESULTS AND DISCUSSION

3.1. Second-order polynomial equation and statistical analysis

Second-order polynomial equations were used to express the empirical relationship between the experimental outcomes and the input variables. The p-value was less than 0.05 ($p < 0.05$), implying that the terms and their interaction were significant. [33-36]

Colour = 7.6999 + 0.11516A - 0.0389417B - 0.0465963C - 0.0625AB - 0.0625AC + 0.0875BC -0.105498A^2 - 0.0171094B^2 - 0.140853C^2.
Taste = 7.26068 + 0.139789A - 0.0512563B - 0.0465963C - 0.0625AB - 0.0625AC + 0.0875BC - 0.060287A^2 - 0.0249316B^2 - 0.130998C^2.
Flavour = 7.12077 + 0.139789A - 0.0512563B - 0.0465963C - 0.0625AB - 0.0625AC + 0.0875BC - 0.0466622 A^2- 0.0113069 B^2 - 0.117373C^2.
Consistency = 6.76068 + 0.139789A - 0.0512563B - 0.0465963C - 0.0625AB - 0.0625AC + 0.0875BC - 0.060287A^2 - 0.0249316B^2 - 0.130998C^2.
Overall Acceptability = 7.21068 + 0.139789A - 0.0512563B - 0.0465963C - 0.0625AB - 0.0625AC + 0.0875BC - 0.060287A^2 - 0.0249316B^2 - 0.130998C^2.

3.1.1. Response 1: COLOR

The ANOVA factors of color response were obtained as shown in Table 6,

Table 6: ANOVA of COLOR Response

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.7293	9	0.0810	6.72	0.0045	significant
A-FM	0.1811	1	0.1811	15.02	0.0038	
B-O	0.0207	1	0.0207	1.72	0.2225	
C-C	0.0297	1	0.0297	2.46	0.1513	
AB	0.0312	1	0.0312	2.59	0.1419	
AC	0.0312	1	0.0312	2.59	0.1419	
BC	0.0613	1	0.0613	5.08	0.0507	
A^2	0.1519	1	0.1519	12.60	0.0062	
B^2	0.0040	1	0.0040	0.3313	0.5790	
C^2	0.2708	1	0.2708	22.45	0.0011	
Residual	0.1085	9	0.0121			
Lack of Fit	0.0485	5	0.0097	0.6473	0.6814	not significant
Pure Error	0.0600	4	0.0150			
Cor Total	0.8379	18				

Table 7 illustrates that the model F-value of 6.72 implies the model is significant. There is only a 0.45% chance that an F-value could occur due to noise. P-values less than 0.0500 ($p < 0.05$) indicate model terms are significant. In this case, A, A^2, and C^2 are significant model terms. The Lack of Fit F-value of 0.65 implies the Lack of Fit is not significant relative to the pure error. Figure 2 depicts the graphical representation of the ANOVA of color response.

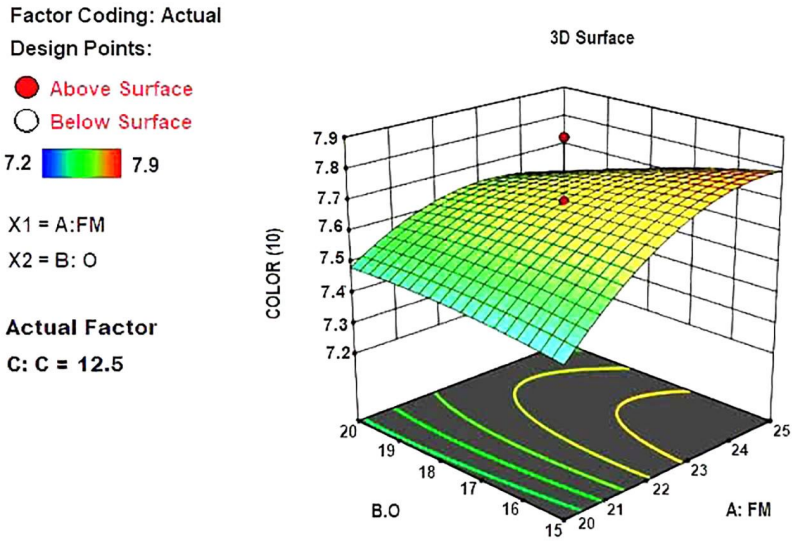

Fig 2: the 3D surface of color response

3.1.2. Response 2: TASTE

The ANOVA factors of taste response were obtained as shown in Table 7,

Table 7: ANOVA of TASTE Response

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.7139	9	0.0793	12.85	0.0004	significant
A-FM	0.2669	1	0.2669	43.22	0.0001	
B-O	0.0359	1	0.0359	5.81	0.0392	
C-C	0.0297	1	0.0297	4.80	0.0561	
AB	0.0312	1	0.0312	5.06	0.0510	
AC	0.0312	1	0.0312	5.06	0.0510	
BC	0.0613	1	0.0613	9.92	0.0117	
A^2	0.0496	1	0.0496	8.03	0.0196	
B^2	0.0085	1	0.0085	1.37	0.2712	
C^2	0.2342	1	0.2342	37.94	0.0002	
Residual	0.0556	9	0.0062			
Lack of Fit	0.0436	5	0.0087	2.90	0.1618	not significant
Pure Error	0.0120	4	0.0030			
Cor Total	0.7695	18				

Table 8 illustrates that the model F-value of 12.85 implies the model is significant. There is only a 0.04% chance that an F-value, could occur due to noise. P-values less than 0.0500 ($p < 0.05$) indicate model terms are significant. In this case, A, B, BC, A^2, and C^2 are significant model terms. The Lack of Fit F-value of 2.90 implies the Lack of Fit is not significant relative to the pure error. Figure 3 depicts the graphical representation of the ANOVA of taste response.

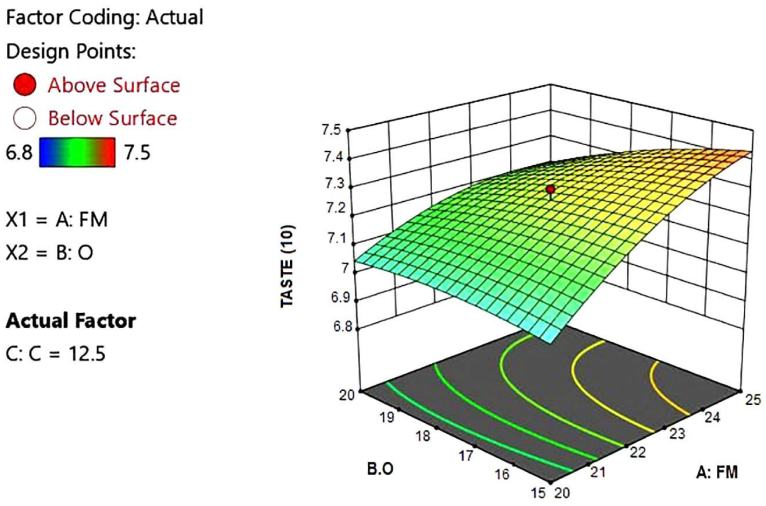

Fig 3: The 3D surface of taste response

3.1.3. Response 3: FLAVOR

The ANOVA factors of flavor response were obtained as shown in Table 8,

Table 8: ANOVA of FLAVOR Response

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.6576	9	0.0731	9.15	0.0015	significant
A-FM	0.2669	1	0.2669	33.42	0.0003	
B-O	0.0359	1	0.0359	4.49	0.0631	
C-C	0.0297	1	0.0297	3.71	0.0861	
AB	0.0312	1	0.0312	3.91	0.0793	
AC	0.0312	1	0.0312	3.91	0.0793	
BC	0.0613	1	0.0613	7.67	0.0218	
A^2	0.0297	1	0.0297	3.72	0.0858	
B^2	0.0017	1	0.0017	0.2186	0.6513	
C^2	0.1881	1	0.1881	23.55	0.0009	
Residual	0.0719	9	0.0080			

Lack of Fit	0.0439	5	0.0088	1.25	0.4254	not significant
Pure Error	0.0280	4	0.0070			
Cor Total	0.7295	18				

Table 9 illustrates that the model F-value of 9.15 implies the model is significant. There is only a 0.15% chance that an F-value could occur due to noise. P-values less than 0.0500 ($p < 0.05$) indicate model terms are significant. A, BC, and C^2 are significant model terms in this case. The Lack of Fit F-value of 1.25 implies the Lack of Fit is not significant relative to the pure error. Figure 4 depicts the graphical representation of the ANOVA of flavor response.

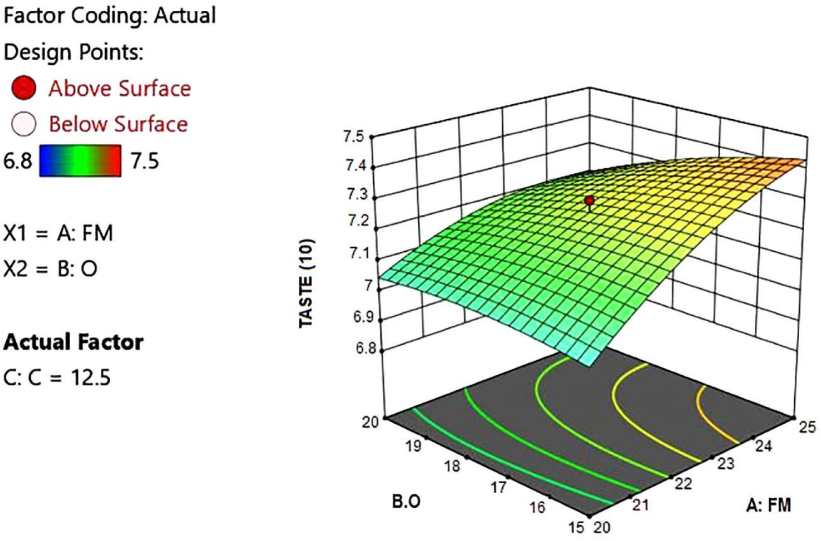

Fig 4: 3d surface of flavor response

3.1.4. Response 4: CONSISTENCY

The ANOVA factors of consistency response were obtained as shown in Table 9,

Table 9: ANOVA of CONSISTENCY Response

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.7139	9	0.0793	12.85	0.0004	significant
A-FM	0.2669	1	0.2669	43.22	0.0001	
B-O	0.0359	1	0.0359	5.81	0.0392	
C-C	0.0297	1	0.0297	4.80	0.0561	
AB	0.0312	1	0.0312	5.06	0.0510	
AC	0.0312	1	0.0312	5.06	0.0510	
BC	0.0613	1	0.0613	9.92	0.0117	
A^2	0.0496	1	0.0496	8.03	0.0196	
B^2	0.0085	1	0.0085	1.37	0.2712	
C^2	0.2342	1	0.2342	37.94	0.0002	
Residual	0.0556	9	0.0062			
Lack of Fit	0.0436	5	0.0087	2.90	0.1618	not significant
Pure Error	0.0120	4	0.0030			
Cor Total	0.7695	18				

Table 10 illustrates that the model F-value of 12.85 implies the model is significant. There is only a 0.04% chance that an F-value could occur due to noise. P-values less than 0.0500 ($p < 0.05$) indicate model terms are significant. In this case, A, B, BC, A^2, and C^2 are significant model terms. The Lack of Fit F-value of 2.90 implies the Lack of Fit is not significant relative to the pure error. Figure 5 depicts the graphical representation of the ANOVA of consistency response.

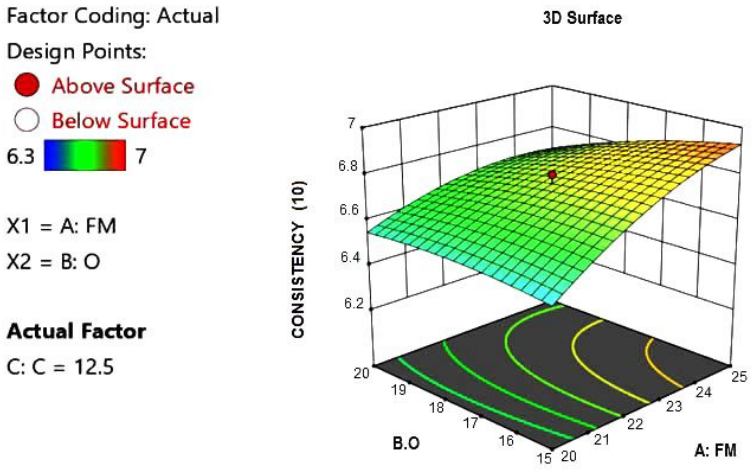

Fig 5: 3d surface of consistency response

3.1.5. Response 5: OVERALL ACCEPTABILITY

The ANOVA factors of overall acceptability response were obtained as shown in Table 10,

Table 10: ANOVA of OVERALL ACCEPTABILITY Response

Source	Sum Of Squares	df	Mean Square	F-Value	p-Value	
Model	0.7486	9	0.0832	14.63	0.0001	significant
A-FM	0.2669	1	0.2669	46.93	< 0.0001	
B-OATS	0.0359	1	0.0359	6.31	0.0308	
C-PEA	0.0297	1	0.0297	5.21	0.0455	
AB	0.0312	1	0.0312	5.50	0.0410	
AC	0.0312	1	0.0312	5.50	0.0410	
BC	0.0613	1	0.0613	10.77	0.0083	
A^2	0.0563	1	0.0563	9.91	0.0104	
B^2	0.0106	1	0.0106	1.87	0.2014	
C^2	0.2558	1	0.2558	44.99	< 0.0001	
Residual	0.0569	9	0.0057			
Lack of Fit	0.0435	5	0.0087	3.26	0.1100	not significant
Pure Error	0.0133	4	0.0027			
Cor Total	0.8055	18				

Table 11 illustrates that the model F-value of 14.63 implies the model is significant. There is only a 0.01% chance that an F-value could occur due to noise. P-values less than 0.0500 ($p < 0.05$) indicate model terms are significant. In this case, A, B, C, AB, AC, BC, A^2, and C^2 are significant model terms. The Lack of Fit F-value of 3.26 implies the Lack of Fit is not significant relative to the pure error. Figure 6 depicts the graphical representation of the ANOVA of the overall acceptability response.

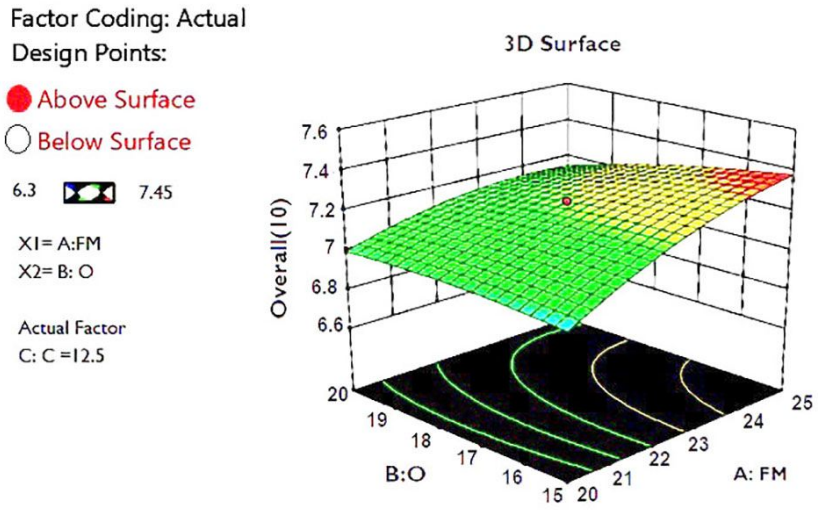

Fig 6: 3d surface of overall acceptability response

3.2. *Sensory evaluation*

The sensory evaluation was conducted for the trials, as mentioned in Section 2.7. All the trials were evaluated, and the average values were noted, as shown in Table 11. [37]

Table 11: Effect of the product on sensory attributes

Attributes	Color	Taste	Flavor	Consistency	Overall acceptability
Trials					
P 1	6.5	5.7	5.8	6.3	6.1
P 2	6.7	5.9	6	6.5	6.3
P 3	7	6.2	6.3	6.5	6.5
T 1	7.7	7.3	7.2	6.8	7.25
T 2	7.8	7.4	7.3	6.9	7.35
T 3	7.9	7.5	7.4	7	7.45
T 4	7.5	7.1	7	6.6	7.05
T 5	7.4	7	6.9	6.5	6.95
T 6	7.3	6.9	6.8	6.4	6.85
T 7	7.6	7.2	7.1	6.7	7.15
T 8	7.2	6.8	6.7	6.3	6.75
T 9	7.5	7.1	7	6.6	7.05
T 10	7.6	7.2	7.1	6.7	7.15

The sensory scores of different attributes of the product were noted. From the above table, Trial 3 scores are higher than other trials.

3.3. *Finalized formulation of supplement mix*

Trial 3 is the finalized formulation for the supplement mix due to their high result in the sensory evaluation, as mentioned in Table 11. The finalized formulation with all ingredients of Trial 3 is mentioned in table 12.

Table 12: Finalized formulation (Trial 3)

Ingredients	Weight (in grams)
Foxtail Millet Powder	25
Oats Powder	15
Green pea protein isolate	10
Carrot powder	15
Pomegranate powder	15
Garlic powder	5
Almond & Cashew nut powder	5
Skim milk powder	10

Fig 7: Finalized supplement mix

3.4. *Physiochemical analysis*

The comparative analysis tests were conducted for Trial 3 composition by the methodologies in IS 12711[20], as mentioned above in Section 2. The results of the main components of physiochemical analysis are moisture, ash, and energy, as mentioned in Table 13.

Table 13: Proximate analysis result of supplement mix			
S. No	Parameters	Unit	Result
1.	Moisture	g/100 g	6.27
2.	Total ash	g/100 g	10.3
3.	Energy	Kcal/100g	360.17

3.5. *Nutritional composition*

The nutritional composition tests were analyzed for the Trial 3 composition by the procedures in IS 12711[20], IS 7219[21], IS 1656[22], IS 5398[23], IS 5399[24], IS 5838[25], IS 5886[26], IS 7529[27] and IS 5949[28], as mentioned above in Section 2. The main parameters focused on in the product are protein and fiber. Besides this, carbohydrates, fat, vitamins, and minerals were also analyzed. The results are mentioned in Table 14.

Table 14: Nutritional composition of supplement mix			
S. No	Parameters	Unit	Result
		Macronutrients	
1.	Carbohydrate	g/100 g	67.57
2.	Total protein	g/100 g	11.5
3.	Fat	g/100 g	4.36
4.	Crude fibre	mg/100g	3.23
		Vitamins	
5.	B1	mg/100g	1.2
6.	B2	mg/100g	1.26
7.	C	mg/100g	55.7
8.	A	µg/100g	370.60
9.	B12	µg/100g	1.98
		Minerals	
10.	Calcium	mg/100g	480
11.	Magnesium	mg/100g	152

3.6. *Shelf-life study*

The shelf-life study was conducted[30,31] for Trial 3 composition, as mentioned in Section 2.23. From the different sensory attributes between D_0 and D_{30}, no significant change was noted, as mentioned in Table 15.

Table 15: Shelf-life difference by sensory characteristics for Trial 3					
Attributes	Color	Taste	Flavor	Consistency	Overall acceptability
Days					
D_0	7.9	7.5	7.4	7	7.45
D_{30}	7.9	7.4	7.3	7	7.4

D_0: Different sensory attributes for Initial day, D_{30}: Different sensory attributes for after 30 days

4. CONCLUSION

A nutraceutical supplement mix for geriatrics was prepared, and its functional and sensory characteristics were studied. This mix was prepared to build the nutrients in older adults, especially, to enhance their gut microbiota. Based on the analysis of nutritional data, the developed combination of supplement mix was highly nutritional. While compared with the RDA chart, the product provided ample protein and energy for daily needs, and this supplement mix can be a good meal replacer as the mix meets the RDA for old-aged adults. The ingredients were chosen by studying their nutritional enhancing characterization on old-aged adults and their prebiotic compositions. The RSM software provided the data for trials, and the final formulation was concluded through sensory data by studying the ANOVA table.

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6. AUTHORS CONTRIBUTION STATEMENT

Saran Kumar S, Nandha Kumar V, and Ragul R conceptualized and designed the study. Dr. Balamurugan P supervised the research, and Saran Kumar S, Ragul R, and Nandha Kumar V collected and organized the data. Saran Kumar S and Nandha Kumar V examined and analyzed the procedures, and Saran Kumar S and Ragul R prepared the draft manuscript. Finally, all the authors reviewed and approved the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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