

Research Paper

Formulation and sensory evaluation of a plant-based essential amino acid-rich food multimix

R Jayashree¹, G Gayathri^{1*}, N Udhayakumar² and CA Kalpana³

¹Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai – 600 116, India

²Department of Paediatrics, Head of Karthikeyan Child Development Unit, Sri Ramachandra Institute of Higher Education and Research, Porur, Chennai – 600 116, India

³Department of Food Science and Nutrition, Deputy Dean, School of Home Science, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu - 641 043, India

*Corresponding author e-mail: gayathri.g@sriramachandra.edu.in

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ABSTRACT

The food multimix approach enhances nutrition via sustainable, accessible, nutrient-rich ingredients, addressing health and environmental concerns. A plant-derived food multimix was formulated using locally available millets, legumes, and nuts (*Paspalum scrobiculatum*, *Panicum sumatrense*, *Glycine max*, *Vigna radiata*, and *Pistacia vera*) to meet 50% of the daily Indian Recommended Dietary Allowances (RDA) for essential amino acids for adults and children. Ingredients underwent preprocessing techniques such as soaking, germination, and roasting. Four variations were formulated among them, Variation IV had the highest amino acid content, meeting 40-70% of the essential amino acid RDA and providing 30-61% of protein needs across age groups. It also contributed 11% of calcium, 25-45% of zinc, and 29-31% of iron requirements. Sensory evaluation of Variation IV in ten South Indian recipes (Idly, Dosa, Onion tomato chutney, Idiyappam, Chapati, Kootu, Sambar, Potato fry, Porridge, Sweet Balls) showed high acceptability among consumers, except for porridge. This multimix enhances nutritional quality, reduces anti-nutritional factors, improves bioavailability and facilitates digestion. This multimix combination provides the essential nutrients and offers a promising solution to undernutrition, aligning with dietary preferences and economic considerations.

Key words: Essential amino acid, Food multi mix, *Glycine max*, *Paspalum scrobiculatum*, *Panicum sumatrense*, *Pistacia vera*, *Vigna radiata*

INTRODUCTION

Despite evolutions in food availability and poverty alleviation, India remains highly susceptible, exhibiting one of the highest rates of undernourishment globally. Various elements contribute to this condition, and the complex interplay among them makes it challenging to identify a single key factor responsible for undernutrition (Sowmya *et al.* 2016). According to the National Family Health Survey-5 (2019-2021), the nutrition profile of Tamil Nadu reveals that among children below 5 years old, the occurrence rates for stunting, wasting, severe wasting, and underweight were 25%, 15%, 5%, and 22% respectively. Additionally, for women aged 15 to 45 years, the occurrence rates of anemia and underweight were 57% and 13% respectively. Around 45% of the rural population in South India failed to meet the World Health Organization's protein recommendations, primarily attributed to inadequate consumption of pulses, meat, and dairy products (Bhutia 2014). Adequate amino acid supply is crucial for neurotransmitter production, hormone

regulation, muscle growth, and cellular processes. These deficiencies are common in economically deprived areas and among elderly adults lacking proper care and severe disorders like Kwashiorkor, Marasmus (Son 2023). The essential amino acids from animal-based proteins meet our body's requirements. However, the rising prevalence of obesity has sparked considerable interest in plant-based protein diets and their impact on both health and the environment, leading to the development of plant-based supplements (Ewy 2022). Therefore, this study focused on the formulation of a food multimix harnessing agricultural resources to address essential amino acid deficiencies among children and adults. The food multimix idea is a food-based approach utilizing traditional food preparation methods and locally obtainable, low-priced, and inexpensive staples (fruits, pulses, vegetables, and legumes) in the preparation of nutrient-enriched multi-mixes (Zotor and Amuna 2008). There is a substantial risk of low-quality protein intake in India, which varies between 4% and 26% in different age groups and

urban and rural locations. It necessitates a healthier consumption of plant-based protein sources, such as legumes, particularly because of their beneficial outcome on the land (Kumari and Sangeetha 2017). Consumption of cereals and legumes holds an essential place in global nutrition, playing a crucial role in traditional food practices across many regions, particularly in the low-income populations in developing countries, where cereals and pulses are considered the best combination for providing essential nutrients (Minocha *et al.* 2017). Considering the points in the aforementioned studies the following ingredients such as *Paspalum scrobiculatum* (Kodo millet), *Panicum sumatrense* (Little millet), *Glycine max* (white soybean), *Vigna radiata* (whole green gram), and *Pistacia vera* (Pistachios) were selected for the development of the food multi mix to address the issue of amino acid deficiency and to increase the protein intake. The objective of the study was to develop a plant-based essential amino acid multimix using conventional pre-processing and cooking techniques, incorporating it into South Indian dishes to meet the 50% of the daily Indian Recommended Dietary Allowance (RDA) of essential amino acids for both adults and children.

METHODS AND MATERIALS

Phase I: Formulation of the supplement

Selection of the ingredients

The ingredients *Paspalum scrobiculatum*, *Panicum sumatrense*, *Glycine max*, *Vigna radiata* and *Pistacia vera* were carefully chosen considering the following four criteria. 1. The food sources that have the highest essential amino acid content were selected from the Indian Food Composition Table (2017) by the National Institution of Nutrition. 2. The choice of ingredients was determined by their bioactive components, including isoflavones, phytic acid, and phenolic compounds, along with their functional attributes such as anti-inflammatory, antioxidant, neuroprotective, and anti-carcinogenic properties (Barreca 2016, Kim 2021, Panda *et al.* 2024, Saleem *et al.* 2023, Venkidasamy 2019). 3. Food groups commonly consumed by the south Indian population. 4. Millets, legumes and nuts were included in the formulation considering their longer shelf life.

Employing pre-processing techniques

Pre-processing techniques such as soaking and germination not only decrease the presence

of antinutrients but also enhance the accessibility of minerals and other nutrients. The nutritional value of grains is impacted by pre-processing methods that preserve the entirety of whole grains as advantageous for one's health (Oghbaei and Prakash 2016). Chosen ingredients were subjected to following conventional cooking processing methods. In soaking, seeds are soaked and the imbibed water activates the intra-cellular pathway which leads to the initiation of the germination process (Venkidasamy 2019). All the selected ingredients were soaked in the water separately for 7 to 8 hours to improve the protein digestibility and to decrease the cooking time. Germination is a recognized and conventional method utilized to enhance the nutritional quality of grains. It has been an ancient method used to soften the kernel structure and to reduce the anti-nutritional factors. The primary objective was to stimulate the hydrolytic enzymes that remain inactive in raw seeds. It also enhances the biosynthetic capacity of the grains, leading to the development of various hydrolytic enzymes. These enzymatic reactions bring about structural modifications and the creation of novel compounds with bioactive properties, contributing to an improved nutritional value of cereal grains (Budhwar 2020). All the soaked ingredients were placed in the air-tight container and maintained at 37°C for 24 hours. Water was periodically sprinkled every 8 hours to ensure proper hydration of the ingredients. Following germination, all the ingredients were exposed to direct sunlight for a day for sun drying. Subsequently, the ingredients were roasted over a medium flame at 40°C for 3 minutes. Finally, all the components were combined and pulverized into a fine powder. The powdered food multi-mix is ready to be added to recipes. It meets

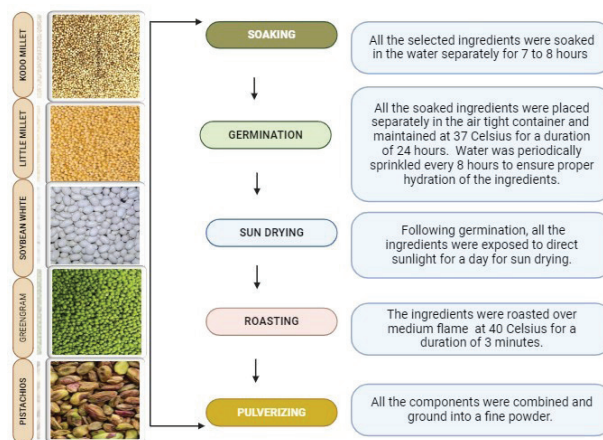


Figure 1: Streamlined process involved in food multi-mix preparation

the safety requirements, for consumption. Figure 1 represents the streamlined process involved in the multi-mix preparation.

Phase II- Development of multi-mix variations and amino acid analysis

Four multi-mix variations, namely I-Millet Max, II-Triple Blend, III-Legume Boost, and IV-Legume Mighty, were meticulously formulated. These names were assigned for the multi-mix variations based on the varying compositions of ingredients. For instance, Variation I, Millet Max, contains the highest ratio of millets, while Variation II, Triple Blend, signifies an equal ratio of millets, legumes, and nuts. Variation III, Legume Boost, has a higher ratio of legumes, whereas variation IV, Legume Mighty, has the highest ratio of legumes compared to the other three samples. The proportions of each ingredient are detailed in Table 1.

Table 1. Multi-mix variations and their corresponding ingredient ratios

Ingredients	Variations	Ingredients Ratio
Kodo Millet/Little Millet/Soyabean	I-Millet Max	3/3/1/1/2
White/Whole Green	II-Triple Blend	2/2/2/2/2
Gram/Pistachios	III-Legume Boost	1/1/3/3/2
	IV-Legume Mighty	0.5/0.5/4/4/1

The rationale behind the development of these four variations is elucidated as follows. Initially, Variation I-Millet Max was formulated and subjected to amino acid content analysis, which indicated a notably lower level of amino acid contents in the 100g of multi-mix powder compared to the Indian RDA. This inadequacy prompted the formation of Variation II-Triple Blend, III-Legume Boost and IV-Legume Mighty, each with varying ingredient proportions. One of our primary objectives was to develop a multi-mix rich in essential amino acids. To achieve this goal, four different variations were developed. However, upon analysis, it was determined that variations I-Millet Max, II-Triple Blend, and III-Legume Boost did not meet the recommended dietary allowance (RDA) for essential amino acids. Therefore, they were excluded from further consideration for analysis involving proximate evaluation and sensory assessment.

Variation IV- Legume Mighty, stood out due to its higher essential amino acid content compared to the other variations. As a result, it underwent additional analysis to evaluate its proximate composition and sensory characteristics. This

decision was made to gain deeper insights into the nutritional and sensory attributes of variation in IV-Legume Mighty.

The quantitative analysis of amino acid content

The quantitative analysis focused solely on the variations (samples), without including any controls as the aim was to assess the amino acid content exclusively within the variations, rather than comparing them to a control group baseline or comparison purposes. Therefore, only variations were included in the amino acid analysis. Amino acid analysis was performed in Ultra High-Pressure Liquid Chromatography N- series with RF-20A amino acid analyzer, which is a highly sensitive, versatile, high speed, reliable with a wide detection range analyser, which was done in a NABL accredited food testing laboratory.

Phase III: Proximate Analysis

Finalized variation IV-Legume mighty multi mix was further analyzed for its nutritional contents (i.e.) calculated energy, carbohydrates, proteins, fats, calcium, iron, zinc, crude fibre, dietary fibre, moisture, and pH content by standardized ISO/ALPL/FD/SOP method at the NABL accredited food lab. Carbohydrates were analyzed by ALPL/FD/SOP/065 where the total carbohydrate content of foods was analyzed directly. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) were determined individually, summed, and subtracted from the total weight of the food. The determination of protein based on total N content by the Kjeldahl method (AOAC IS 7219). Fat was analysed by A gravimetric method (AOAC 21st Edition 2016, 920.85).

Phase IV: Assessing the sensory characteristics of dishes infused with the sample IV -Legume Mighty formula

The finalized multi-mix Variation IV-Legume Mighty approximately 5 to 10g, was uniformly incorporated into single portions of ten traditional South Indian recipes such as 2 Idles (Steamed rice cakes), 2 Dosas (Rice and Lentil Pancakes), 1 Idiyappam (Steamed rice noodles), 2 Chapatis (Indian wheat Flatbread), 1 serving of Onion Tomato Chutney (Spicy Tomato Onion Sauce), 1 serving of Potato Fry (Spicy Pan Potatoes), 100 ml of Sambar (Lentil Stew), 1 serving of Kootu (Mixed Vegetable Stew), 150 ml of Porridge, and 2 Sweet Balls (Sweet bites) weighing approximately 20 g each and

all the recipes were evaluated for their sensory characteristics. The sensory evaluation took place at the Food Sensory Laboratory of Avinashilingam University in Coimbatore. Each of the ten recipes was presented in two variations: firstly, as ten standard recipes serving as controls without the inclusion of the multi-mix, and secondly, as ten sample recipes incorporating the multi-mix. The Food Sensory Laboratory was equipped with specialized lighting and isolated chambers, which provided controlled conditions for food evaluation. These amenities guaranteed that participants could evaluate the recipes without any external impacts or biases, preserving the integrity of the sensory evaluation process.

Thirty participants participated to evaluate the various sensory characteristics, such as appearance, flavour, colour, taste, texture, and overall acceptability of the recipes, using a 9-point hedonic scale which is a well tried and tested in consumer research for capturing liking data. The 9-point hedonic scale contains scores ranging from 1 to 9. Participants gave scores based on their individual preferences and liking levels for each recipe. The objective was to comprehensively evaluate the sensory properties of the recipes and discern any differences arising from the inclusion of the finalised multi-mix. The scores provided by participants for each sensory attribute of all ten recipes were statistically analyzed to determine the significance between the sample and standard recipes.

RESULTS AND DISCUSSION

Four sample variations, namely I-Millet Max, II-Triple Blend, III-Legume Boost, and IV-Legume Mighty were analyzed for their amino acid composition utilizing Ultra High-Pressure Liquid Chromatography N-series with RF-20A amino acid analyser. Table 2 displays the amino acid content present in the four distinct multi-mix variations. Table 3 compares the levels of essential amino acids found in sample variation IV- Legume Mighty with their corresponding RDAs for both adults and children. Based on the above mentioned data in the Table 2, it was evident that variation IV- Legume Mighty contains a significant amount of essential amino acids, leading to its selection for the subsequent proximate analysis. The Table 4 represents the analysis method and macro, micronutrients present in the 100 g of the developed multi mix. The percentage of Indian RDA of different nutrients fulfilled by the 100 g of the supplement

specifically designed for various age groups (Table 5).

Table 2. Amino acid content of the four multi-mix variations

Essential amino acids	Variation I (g) Millet Max	Variation II (g) Triple Blend	Variation III (g) Legume Boost	Variation IV (g) Legume Mighty
Valine	0.13	0.37	0.53	0.78
Isoleucine	0.10	0.34	0.20	0.52
Leucine	0.62	0.43	0.63	0.62
Lysine	0.18	0.18	0.18	0.61
Methionine	0.13	0.60	0.13	0.68
Histidine	0.70	0.95	0.70	0.60
Phenylalanine	0.39	0.18	0.39	0.58
Tryptophan	0.11	1.75	0.21	0.11
Threonine	0.36	1.08	0.86	1.12

Table 3. Amino acid content comparison per 100g of multi mix among adults and children

Essential amino acids	Multi mix (mg)	Male RDA (mg)	Female RDA (mg)	Children RDA (mg)
Valine	0.7	1.7	1.4	0.7
Isoleucine	0.5	0.7	0.5	0.5
Leucine	0.6	0.9	0.7	1.1
Lysine	0.6	0.8	0.6	0.8
Methionine	0.6	0.9	0.7	0.4
Histidine	0.6	0.8	0.6	0.3
Phenylalanine	0.5	0.9	0.7	0.7
Tryptophan	0.1	0.2	0.1	0.1
Threonine	1.1	1.9	1.5	0.4

Table 4. Proximate analysis findings for variation IV-Legume Mighty Multimix

Nutrients	Analysis method	Results
Carbohydrate	ALPL/FD/SOP/065	66.7 g
Protein	IS: 7219:1973	18.1 g
Fat	AOAC 21 st Edition 2016, 920.85	4.3 g
Energy	ALPL/FD/SOP/076	378 kcal
Iron	ALPL/FD/SOP/068	4.9 mg
Calcium	IS: 5949: 1990	70 mg
Zinc	AOAC 22nd Edn, 2023	54.9 mg/kg
Dietary fibre	AOAC 22nd Edn, 2023	5.8 %
Crude fibre	AOAC 21 st Edition 2016, 962.09	2.8 g
Moisture	FSSAI Lab Manual	7 g
pH	FSSAI Lab Manual	6

Descriptive statistics

Table 6 compares the overall acceptability scores of standard and sample recipes. Various south Indian recipes, including Idly (Steamed rice cakes), Dosa (Rice and Lentil Pancakes), Onion

Tomato Chutney (Spicy onion tomato sauce), Idiyappam (Steamed rice noodles), Chapati (Indian wheat Flatbread), Kootu (Mixed Vegetable Stew), Sambar (Lentil Stew), Potato Fry (Spicy Pan Potatoes), Porridge, and Sweet Balls (Sweet bites) were prepared both with (sample recipes) and without (Standard recipes) the formulated multimix powder. Each sensory attribute, including appearance, colour, taste, texture, and flavour, contributes to food evaluation. However, the overall acceptability score summarizes participants' overall liking or satisfaction with the food product. This score integrates all sensory aspects and serves as the ultimate measure of the food's appeal. Hence, the overall acceptability scores given by the 30 participants are presented in Table 6.

Statistical analysis of overall acceptability scores for ten recipes

Acceptability scores for ten recipes were analyzed using Kruskal-Wallis' test to compare their distribution and significance against a standard recipe. Scores ranged from 1 to 9, with frequency recorded for both sample and standard recipes. Table 7 presents the test results, showing any significant differences between standard and sample recipes. Table 3 provides a comparison between the RDA of essential amino acids and the essential amino acids content present in the 100g of multimix designed for males, females, and children. Across all the categories, it was evident that the amino acid levels in the food multimix

generally satisfy approximately half of the daily requirements. However, it was significant that in the case of children, the levels of threonine, valine, and methionine in the supplement exceeded the recommended daily intake. Adults will be recommended to consume 100g of the supplement to fulfill half of their daily requirements, while children will be advised to consume 50g of the supplement to meet their daily needs along with their food.

From Table 5 it was evident that the formula meets 30%, 33%, and 61% of the protein requirement for men, women, and 7-year-old children, respectively. This suggests it as a promising protein source. Additionally, it met 11% of the calcium requirement and 28%, 23%, and 30% of the iron requirement for men, women, and children, respectively. The multi-mix fulfilled 45%, 25%, and 33% of the zinc requirement for men, women, and children, respectively. A study was conducted in Indonesia, where a food multimix was formulated and used in an intervention among stunted but not wasted children to enhance their micronutrient and amino acid intake. The study concluded that combining complementary feeding with a food multimix made from underutilized locally available food sources significantly improved the overall nutrient intake of the children (Wirawan *et al.* 2022).

The results of the Kruskal-Wallis one-way analysis of variance indicated that all recipes, except for Porridge, exhibited statistical significance in terms of sensory attributes and overall acceptability. Despite Porridge showing a noticeable difference

Table 5. Comparison of Indian recommended dietary allowance (RDA) with 100g of multi mix: nutrient percentage met among men, women, and children

Nutrients	Nutrients present in the 100g of multi-mix	Men		Women		Children	
		RDA	% of RDA met	RDA	% of RDA met	RDA	% of RDA met
Energy (K.cal)	378	2730	14%	2230	17%	1690	22%
Protein (g)	18.1	60	30%	55	32%	29.5	61%
Fat (g)	4.3	30	14%	25	17%	30	14%
Iron (mg)	4.9	17	28%	21	23%	16	30%
Calcium (mg)	70	600	11%	600	11%	600	11%
Zinc (mg)	5.4	12	45%	21	25%	16	33%

Note: The table provides a comparison of the Indian Recommended Dietary Allowance (RDA) for various nutrients among moderately active men, women, and children aged 7 to 9 years.

Table 6. Comparison of overall acceptability scores of standard and sample recipes

Recipes	Overall acceptability M±SD	
	Standard	Sample
Idly (Steamed rice cakes)	7.4 ± 1.1	7.2 ± 1.0
Dosa (Rice and Lentil Pancakes)	7.0 ± 1.5	7.0 ± 1.5
Onion Tomato chutney (Spicy onion tomato sauce)	7.7 ± 0.9	7.6 ± 0.9
Idiyappam (Steamed rice noodles)	7.4 ± 0.7	7.2 ± 0.8
Chapati (Indian wheat Flatbread)	7.5 ± 1.5	7.1 ± 1.3
Kootu (Mixed Vegetable Stew)	7.7 ± 0.9	10 ± 14.5
Sambar (Lentil Stew)	7.7 ± 1.0	7.7 ± 0.8
Potato fry (Spicy Pan Potatoes)	7.7 ± 0.6	7.9 ± 0.8
Porridge	7.4 ± 0.9	7.6 ± 1.0
Sweet Balls (Sweet bites)	7.8 ± 1.1	7.8 ± 1.0

Note: Standard- Standard recipes served as controls, without the inclusion of the multi mix, Sample- Sample recipes included the food multi mix, M-Mean, SD- Standard deviation

Table 7. Kruskal-Wallis test results for various recipes

S. No.	Recipes	Category	Standard	Sample	Kruskal-Wallis's test Statistics	Chi-square distribution	p-value
1	Idly	5	0	1	9.98	3df	0.003**
		6	5	3			
		7	7	5			
		8	7	12			
		9	11	9			
2	Dosa	5	2	2	15.88	4 df	0.003**
		6	5	5			
		7	7	11			
		8	11	9			
		9	5	3			
3	Onion Tomato Chutney	5	0	0	8.91	3df	0.03*
		6	3	7			
		7	9	9			
		8	16	14			
		9	2	0			
4	Idiyappam	1	1	1	14.08	4df	0.007**
		6	3	1			
		7	6	17			
		8	14	11			
		9	6	0			
5	Chapati	1	1	1	18.7	5df	0.002**
		5	3	3			
		6	3	3			
		7	10	11			
		8	10	9			
6	Kootu	5	0	1	10.4	3df	0.015*
		6	3	3			
		7	9	8			
		8	12	11			
		9	6	7			
7	Sambar	6	1	2	9.19	3df	0.027*
		7	8	6			
		8	18	15			
		9	3	7			
		6	3	2			
7	15	14					
8	4	9					
9	8	5					
9	Porridge	5	2	2	7.65	4df	0.105 ^{NS}
		6	1	1			
		7	10	10			
		8	15	11			
		9	2	6			
10	Sweet balls	5	1	1	14.609	3df	0.002**
		6	0	2			
		7	12	9			
		8	11	12			
		9	6	6			

Note: Standard- Standard recipes served as controls, without the inclusion of the multi-mix. Sample- Sample recipes included the food multi-mix, Category- The acceptability score ranges from 1 to 9. Kruskal-Wallis Test Statistics- compares the medians of multiple groups to determine significant differences, Chi-Square Distribution- The degrees of freedom (df) associated with the Kruskal-Wallis's test statistic, based on the number of groups being compared, p-value: ** represents highly significant, * represents the significant, ^{NS} represents nil significant.

in scores, it did not reach statistical significance. Notably, idly (Steamed rice cakes), Dosa (Rice and Lentil Pancakes), Onion Tomato Chutney (Spicy onion tomato sauce), Chapati (Indian wheat flatbread, and sambar (Lentil Stew) demonstrated high significance, suggesting distinct preferences among participants for these recipes. On the other hand, Sweet Balls (sweet bites), Potato Fry (Spicy Pan Potatoes), Idiyappam (Steamed rice noodles), and Kootu (Mixed Vegetable Stew), showed significant

differences, implying that these recipes were also equally well-accepted by the participants. Overall, the Chi-square test highlighted the varying degrees of acceptance and preference for the different recipes in the study. Similar results were observed in the study conducted by Zotor *et al.* (2018) in Sub-Saharan Africa, where food multi-mixes incorporated into biscuits, soups, and porridge received the highest scores in sensory evaluations.

CONCLUSION

Despite various government efforts, undernutrition remains a challenge in developing countries like India, particularly affecting children and women. To address this issue, sustainable dietary supplements are gaining importance. The novel essential amino acid-rich multi-mix introduced in this study offers a safe, plant-based alternative for combating malnutrition, especially among children with protein-energy malnutrition. This multi-mix is safe, uses well-known and commonly consumed ingredients, and is acceptable to vegetarian populations. Its versatility is enhanced by integration into ten different South Indian recipes, increasing acceptability. Cost-effective and easy to prepare, it is accessible to lower and middle-income groups. Pre-processing techniques enhance nutritional quality, reduce anti-nutritional content, and aid digestion. Combining millets, legumes, and nuts, the food multi-mix provides essential nutrients, including crucial micronutrients. The multi-mix can be distributed through the Public Distribution System (PDS) and extended to children, pregnant women, and nursing mothers via Balwadi and Anganwadi centers. This approach fosters healthy development and inclusive wellness, prioritizing health without financial barriers.

In conclusion, this study offers a promising solution to undernutrition, presenting a safe, well-accepted, and nutritionally rich food multimix that aligns with dietary preferences and economic considerations in regions facing persistent malnutrition.

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