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Development and Evaluation of Immune Boosters Based Health Plus for HIV/AIDS

Pa. Raajeswari[1]* and N. Bhooma[2]

[1]Assistant Professor, Department of Food Science and Nutrition, Avinashilingam Institute for Homescience and Higher Education for Women , Coimbatore-43, India; raajraaeshwari@gmail.com
[2]Professor, Department of Food Science and Nutrition ,Arinashilirjam Institute for Home Science and Higher Education forwomen, Coimbatore-43; India.

Abstract

An ideal food for prevention and correction of nutritional inadequacies should be of high nutritive value, acceptable, readily available at low price, familiar to the community and have good tolerance both in good health and illness. Development of supplements using low cost locally available indigenous foods familiar to the community, especially women, has been one of the strategies proven to be effective in improving the health status of the community. Keeping this in view, the present study was undertaken with the main objective to identify the locally available foods rich in immune boosters, modulators and regulators, and to formulate different five health mixes coded as HMI, HMII, HM III, HM IV and HM V. To identify the best combination and proportion of ingredients, six variations were formulated from each health mix making the total to thirty. All the thirty variations of the health mixes were given different code numbers. By applying five point rating scale and DMR test, the highly acceptable mix was selected. The results showed that among the best varieties from each health mix, variations from each health mix variation 5 of health mix V got the highest score in taste and colour, maximum nutrient content, minimum moisture content and peroxide value and less total bacterial count and hence variations of health mix V was adjusted to be the best and named as HEALTH PLUS. The findings of the study revealed that the ingredients namely wheat, soy flour, wheat germ, tomato, beet root, sun flower seeds, and jaggery which are available at our doorsteps are foods rich in immune boosters, modulators and regulatory to improve the immune level of the HIV positive women.

Keywords: Bamboo Seed Flour, Central Composite Rotatable Design, Pasting Properties, Rapid Visco Analyser, Standard 1 Profile

1. Introduction

The relationship between nutrition and HIV is a vicious cycle, similar to the relationship between nutrition and other infections. Poor nutritional status is one of the major complications of HIV and a significant factor in full-blown AIDS. A well-balanced diet and micronutrient supplementation seem to be warranted to ensure optimal health and survival particularly in HIV-infected women [1]. Provision of simple, inexpensive micronutrient supplements as an adjunct to Highly Reactive Anti Retroviral Therapy (HAART) may have several cellular and clinical benefits, such as reduction in mitochondrial toxicity, oxidative stress and improvement in immune reconstitution [2]. An ideal food for prevention and correction of nutritional inadequacies should be of high nutritive value, acceptable, readily available at low price, familiar to the community and have good tolerance both in good health and illness [3], [4]. Development of supplements using low cost locally available indigenous foods familiar to the community especially women, has been one of the strategies proven to be effective in improving the health status of the community [5]. Hence the present study was undertaken to develop and evaluate a immune boosters based health mix for feeding HIV positive women.

*Author for correspondence

2. Materials and Methods

2.1 Formulation and Standardisation of Health Mixes

2.1.1 Identification and Selection of Foods Rich in Immune Boosters, Modulators and Regulators

Food supplementation is one of the effective ways of improving the health status of affected HIV/AIDS positive women by means of increasing the CD4 cell count and hence decreasing the chances of opportunistic infections. Keeping all these points in mind the investigator formulated different health mixes consisting of food immune boosters, modulators and regulators chosen from five food groups Foods namely wheat, corn, soy, soy protein isolate, wheat germ, tomato, beetroot, carrot, amla, garlic, onion , peanuts, sunflower seeds and jaggery were identified as foods that improve immune function. The identified food ingredients rich in immune boosters, modulators and regulators are given in plate 1. The identified foods along with their functional principles responsible for improving the immune system in People Living With HIV/AIDS is given in Table 1..

2.1.2 Formulation and Evaluation of Different Health Mixes

2.1.2.1. Formulation of Different Health Mixes

The selected ingredients (indicated in the Table 1) were processed and formulated into different health mixes. The preliminary preparation of ingredients such as cutting, peeling, washing and sprouting were adopted to enhance the palatability and acceptability of the food ingredients.

Whole wheat was germinated for 24 hours to increase the nutrient content. Corn flour, soy flour, wheat germ and soy protein isolate were purchased as such to incorporate in the health mix. Carrot, beetroot and amla were thoroughly washed, scrapped and shade dried for a week. Tomato was thoroughly washed and cut into small pieces. Fresh thulasi leaves were obtained from the farm. Edible portions of onion and garlic were chopped into small pieces.

Clean sunflower seeds and peanuts were used. The above mentioned ingredients were allowed to shade dry in a plastic sheet and were turned off frequently at regular intervals to ensure proper drying. The dried ingredients were individually roasted at suitable temperature to enhance the aroma and shelf-life. Roasting technique was used in the processing of cereals, pulses and oil seeds [6] to improve availability. The details regarding the pre-treatment and processing techniques followed for the different food ingredients is outlined in the Figure 1. The processed ingredients were ground into fine powder (Plate 4) and stored in air tight containers separately. Five Health Mixes (HM) were prepared using the processed food ingredients and coded as HM I, HM II, HM III, HM IV, and HM V. To identify the best combination and proportion of ingredients six variations were formulated from each health mix making the total to thirty. Finally thirty formulations were evolved and the detail of the formulations is presented in Tables 2 to 6.

2.1.2.2 Acceptability Trial

The formulated health mixes were intended for feeding HIV positive women to improve their nutritional and health status. Organoleptic evaluation of the thirty variations was carried out by 10 panels of judges. All the thirty variations of the health mixes were given different code numbers. Each panel of judges were given 25 gms of health mixes in the form of ladoos, prepared using 25 gms of health mixes with hot water to assess the quality factors such as colour, flavour, texture and taste. The overall acceptability was assessed using a five point rating scale.

2.1.2.3 Shelf Life Assay of the Health Mixes

The health mixes were packed in heat sealed low density polyethylene bags (200 gauges) and stored at room temperature between 27 and 35°C with relative humidity of 70-85 per cent for three months for their keeping quality evaluation. The shelf life of the health mixes was assessed through organoleptic parameters, moisture content [7], peroxide value (Sadasivam, 1997) and total bacterial count by pour plate method in comparison with the fresh formulations.

3. Results and Discussion

3.1.1 Organoleptic Evaluation of Health Mixes

Five health mixes with six variations each making the total of 30 variations were developed and subjected to organoleptic evaluation and the scores are presented in Table 7.

Table 1. The identified foods along with their functional principles

S.No	Food Immune Boosters, Modulators and Regulators	Functional Principles
1	Wheat	Minerals, antioxidants (tocotrienols, selenium, phenolic acid and phytic acid), lignans, phytonutrients, water – soluble, fat soluble vitamins
2	Corn	Cryptoxanthian, thiamin, pantothenic acid, folate and iron
3	Soy	High quality protein, isoflavones, aminoacids, terpernoids, iron, calcium, and B-vitamins
4	Soya protein isolate	High quality protein, isoflavones, aminoacids, terpernoids, iron, calcium, and B-vitamins
5	Wheat germ	Antioxidants-isoflavones, peptides, carbohydrates, fatty acids, coenzymes, amines, poly amines, saponins, terpenoids.
6	Tomato	Vitamin – A, C , E, zinc and lycopene, phosphorus, sulphur, potassium and zinc
7	Beetroot	Vitamin A,C, E, di-o-gallolyl-d glucose, digalleo acid, betaine, choline, folic acid and iodine
8	Carrot	Beta-carotene, vitamin-K, E and C
9	Amla	Vitamin-C, calcium, phosphorus and iron
10	Thulasi	Flavonoids, eugenol, iron, calcium, vitamin-A and vitamin-K
11	Garlic	Minerals, antioxidants (tocotrieonols, selenium, phenolic acid and phytic acid), lignans, phytonutrients, water soluble and fat soluble vitamins.
12	Onion	Antioxidants-isoflavones, peptides, carbohydrates, fatty acids, coenzymes, amines, poly amines, saponins and terpenoids.
13	Peanuts	Unsaturated fats, protein, fiber, vitamin – E, selenium, zinc, folate, iron and phytochemicals.
14	Sunflower seeds	Unsaturated fats, protein, fiber, vitamin – E, selenium, zinc, folate, iron and phytochemicals.
15	Jaggery	Medicinal sugar- minerals, iron, vitamins mainly manganese and selenium

Table 2. Health mix I

S.No.	Ingredients	Variations (g)					
		1	2	3	4	5	6
1.	Wheat	20	10	30	25	20	10
2.	Soy flour	20	10	30	25	20	10
3.	Beetroot	20	30	10	15	10	20
4.	Tomato	20	30	10	15	10	20
5.	Sunflower seeds	10	10	10	10	20	20
6.	Jaggery	10	10	10	10	20	20

Table 3. Health mix II

S.No.	Ingredients	Variations (g)					
		1	2	3	4	5	6
1.	Wheat	20	25	30	20	30	10
2.	Soy protein isolate	20	25	30	20	30	10
3.	Corn flour	10	10	10	15	5	20
4.	Thulasi	15	10	10	15	5	20
5.	Carrot	15	10	10	15	10	20
6.	Jaggery	20	20	10	15	20	20

Table 4. Health mix III

S.No.	Ingredients	Variations (g)					
		1	2	3	4	5	6
1.	Wheat	20	15	25	10	15	20
2.	Soy flour	20	20	15	20	15	20
3.	Corn flour	10	15	20	20	15	20
4.	Thulasi	2.5	2	5	5	10	5
5.	Carrot	5	2.5	2.5	5	10	5
6.	Peanuts	5	7.5	2.5	12.5	10	10
7.	Sunflower seeds	5	6	2.5	5	5	5
8.	Amla	2.5	2	5	2.5	5	5
9.	Jaggery	30	30	22.5	20	15	10

The proportions were transformed into angles by applying sinx and angular transformation to make the variable (proportion) into a normal variable. Analysis of variance technique was then employed to determine the priority of the groups. The F test turned out to be non-significant, since the group priority is a necessity in the follow-up study where the supplementation aspect has to go on with a particular group.

Table 5. Health mix IV

S.No.	Ingredients	Variations (g)					
		1	2	3	4	5	6
1.	Wheat	20	15	10	5	10	15
2.	Soy flour	15	20	15	10	10	5
3.	Beet root	10	5	10	15	10	15
4.	Sunflower seeds	5	10	15	20	15	20
5.	Garlic	15	10	5	10	15	20
6.	Onion	15	10	5	10	15	10
7.	Tomato	10	15	20	15	10	5
8.	Carrot	10	15	20	15	15	10

Table 6. Health mix V

S.No.	Ingredients	Variations (g)					
		1	2	3	4	5	6
1.	Wheat	15	15	10	20	15	15
2.	Soy flour	20	10	20	20	15	5
3.	Wheat germ	15	20	20	15	15	15
4.	Beetroot	15	20	15	15	15	5
5.	Tomato	15	15	20	10	15	20
6.	Sunflower seeds	15	10	15	10	10	20
7.	Jaggery	5	10	10	10	15	20

Therefore the DMR test was applied for the same data, where the GP again turned to be not being a significantly different one. Based on the inferences, taking the variables under analysis as a robust free variable the Fried Man's two- way analysis of the variance taking the group as the treatment and experimental individuals as conditions, has been thought as the correct methodology and the results are as follows. As far as health mix I is concerned variation 1 obtained the scores 2.3, 3.2, 3.2, 3 for the characteristics colour, flavour, texture and taste respectively, with the overall acceptability score of 11.7 out of the maximum score 20. Similarly variation 2 received the scores 1.4, 3, 4.1 and 2.7 for the various characteristics with the overall acceptability score of 11.2. Variation 3 obtained 3.5, 3.6, 3.6, 3.7 for the various characteristics with the overall acceptability score of 14.4 out of the maximum score of 20. Variation 4 obtained 3.9 for colour out of 5, 2.8 for flavour, 4.3 for texture and 3.8 for taste, with the maximum overall acceptability score of 14.8. Variation 5 obtained 3.8 for colour, 3.1 for flavour, 3.4 for texture and 3.6 for taste with the overall acceptability score of 14.0. Variation 6 obtained 1.9 for colour, 3.1 for flavour, 3.6 for texture and 3.4 for taste with the overall acceptability score of 12 out of the maximum score 20. In health mix II, variation 1 secured the scores 3, 2.5, 3 and 3.1 for the various characteristics with the overall acceptability score of 11.6 , variation 2 obtained 2.6, 2.8, 2.9 and 2.1 for the characteristics with the overall acceptability score of 10.4 out of the maximum score 20. Variation 3 obtained the scores 2.9, 4, 3.9 and 3.3 for characteristics such as colour, flavour, texture and taste respectively with the overall acceptability score of 14.1 out of the maximum score 20. Variation 4 and 5 received 2.7, 2.4 for colour 3.2, 3.4 for flavour 4, 3.4 for texture and 3.3, 3.1 for taste with the overall acceptability scores of 9.4 and 12.3 respectively. Variation 6 obtained 2.5 for colour out of 5, 3 for flavour, 3.1 for texture and 3.2 for taste, with the overall acceptability score of 11.8.

As far as health mix III is concerned variation 1 obtained scores 2, 3.2, 3.3, 3.1 for the characteristics colour, flavour, texture and taste respectively, with the overall acceptability score of 11.6 out of the maximum score 20. Similarly variation 2 received the scores 1.2, 3.2, 3.3 and 3.1 for the various characteristics with the overall acceptability score of 9.9. Variation 3 obtained 3.6, 3.5, 2.6, 2.7 for these characteristics with the overall acceptability score of 13.4 out of the maximum score of 20. Variation 4 obtained 2.8 for colour out of 5, 3.5 for flavour, 4 for texture and 2.2 for taste, with the maximum overall acceptability score of 12.5. Among the six, variations 5 and 6 received 2.5, 2.7 for colour, 3, 3.1 for flavour, 3.5, 3.9 for texture and 2.7, 2.9 for taste with the overall acceptability score of 11.7 and 12.6 respectively.

In health mix IV, variation 1 received the scores 2.3, 2.1, 3.1 and 2 for the various characteristics with the overall acceptability score of 9.5. Variation 2 obtained 3.8, 3.6, 3.1 and 2.6 for the characteristics with the overall acceptability maximum score of 13.1 out of the maximum score 20. Variation 3 obtained scores 3.3, 2.1, 3.2 and 2.6 for characteristics such as colour, flavour, texture and taste respectively with the overall acceptability score of 11.2 out of the maximum score 20. Variation 4 and 5 received 3, 3.4 for colour; 2.8, 2.6 for flavour; 3, 4 for texture and 2.2, 3 for taste with the overall acceptability scores of 11 and 13 respectively. Variation 6 obtained 3.1 for colour out of 5, 3.4 for flavour, 2.9 for texture and 2.6 for taste, with the overall acceptability score of 12. As far as health mix V is concerned variation 1 obtained scores 3.9, 3.5, 3.6, 3.7, 3.1 for the characteristics colour, flavour, texture and taste respectively, with the overall

Table 7. Organoleptic evaluation of health mixes (Maximum Score: 20)

Variations (5)	Colour (5)	Flavour (5)	Texture (5)	Taste (5)	Overall Acceptability (20)
Health Mix I					
1	2.3	3.2	3.2	3	11.7
2	1.4	3	4.1	2.7	11.2
3	3.5	3.6	3.6	3.7	14.4
4	**3.9**	**2.8**	**4.3**	**3.8**	**14.8**
5	3.8	3.1	3.4	3.6	14.0
6	1.9	3.1	3.6	3.4	12
Health Mix II					
1	3	2.5	3	3.1	11.6
2	2.6	2.8	2.9	2.1	10.4
3	**2.9**	**4**	**3.9**	**3.3**	**14.1**
4	2.7	3.2	4	3.3	9.4
5	2.4	3.4	3.4	3.1	12.3
6	2.5	3	3.1	3.2	11.8
Health Mix III					
1	2	3.2	3.3	3.1	11.6
2	1.2	2.5	3.3	2.9	9.9
3	**3.6**	**3.5**	**2.6**	**2.7**	**13.4**
4	2.8	3.5	4	2.2	12.5
5	2.5	3	3.5	2.7	11.7
6	2.7	3.1	3.9	2.9	12.6
Health Mix IV					
1	2.3	2.1	3.1	2	9.5
2	**3.8**	**3.6**	**3.1**	**2.6**	**13.1**
3	3.3	2.1	3.2	2.6	11.2
4	3	2.8	3	2.2	11
5	3.4	2.6	4	3	13
6	3.1	3.4	2.9	2.6	12
Health Mix V					
1	3.9	3.5	3.6	3.7	14.7
2	3.7	3.6	3.6	3.7	14.6
3	3	3	2.3	2.8	11.1
4	2.1	2.9	2.9	2.5	11.4
5	**4.3**	**3.8**	**4.3**	**4.2**	**16.6**
6	2	2.9	1.9	3.6	10.4

acceptability score of 14.7 out of the maximum score 20. Similarly variation 2 received the scores 3.7, 3.6, 3.6, 3.7 for the various characteristics with the overall acceptability score of 14.6. Variation 3 obtained 3, 3, 2.3, 2.8 for the characteristics with the overall acceptability score of 11.1 out of the maximum score of 20. Variation 4 obtained 2.1 for colour out of 5, 2.9 for flavour, 2.9 for texture and 2.5 for taste, with the maximum overall acceptability score of 11.4. Among the six, variations 5 and 6 received 4.3, 2 for colour; 3.8, 2.9 for flavour; 4.3, 1.9 for texture and 4.2, 3.6 for taste with the overall acceptability score of 16.6 and 10.4 respectively.

Ten panellists evaluated the thirty variations of health mixes. Among thirty variations, one variation which got the highest score from each type of health mix was selected and subjected to further analysis.

3.1.2 Overall Acceptability Scores of Health Mixes

Table 8 presents the mean overall acceptability scores of health mixes.

Table 8. Overall acceptability scores of health mixes

Variations	Health Mix I	Health Mix II	Health Mix III	Health Mix IV	HealthMix V
1	11.7	11.6	11.6	9.5	14.7
2	11.2	10.4	9.9	**13.1**	14.6
3	14.4	**14.1**	**13.4**	11.2	11.1
4	**14.8**	9.4	12.5	11	11.4
5	14.0	12.3	11.7	13	**16.6**
6	12	11.8	12.6	12	10.4

When the overall acceptability of the scores of variations were compared for the five health mixes, variation 4 of health mix I, variation 3 of health mix II, variation 3 of health mix III, variation 2 of health mix IV and variation 5 of health mix V received the highest scores when compared with their respective counterparts. One variation from each health mix, which got the highest score was selected and subjected to moisture content and microbial assay.

3.1.3. Shelf Life of the Health Mixes

3.1.3.1 Moisture Content and Peroxide Value of the Selected Five Variations

Moisture content is a critical index for prevention of spoilage. Peroxide value indicates the lipid oxidation. The details regarding moisture content and peroxide value of the selected five variations are given in the fresh form and the sample preserved for ninety days (Table 9).

It was observed that there was an increase in the moisture content of all the five variations on storage. But the increase in the moisture content was within the safe levels (15.1 per cent) given by FAO/WHO [8]. The minimum gain in the moisture may be due to the use of low density polyethylene bags for storing [9]. Similarly the peroxide value in the five variations varied between 0.01 and 0.02 meq/kg. On storage it increased slightly but was within the safe level suggested by FAO/WHO [10], indicating that the health mixes did not undergo rancidity on storage for ninety days.

3.1.3.2 Microbial Assay

Combination of foods by bacterial load and mould could result in unacceptable products [11]. Microbial count in the health mixes on storage (room temperature) was compared with the fresh formulations and the details are given in Table 10.

Table 9. Moisture content and peroxide value of the selected five variations

VARIATIONS	MOISTURE (PERCENT)	PEROXIDE VALUE (meq/kg)
HM I VAR 4 A	5.82	0.010
B	5.80	1.017
HM II VAR 3 A	6.15	0.010
B	6.19	0.013
HM III VAR 3 A	5.19	0.010
B	5.22	0.014
HM IV VAR 2 A	5.55	0.020
B	5.59	0.028
HM V VAR 5 A	4.66	0.010
B	4.71	0.050

HM – Health Mix, VAR – Variation
A – Fresh Sample B – 90 days after storage

Table 10. Total bacterial count in the selected five variations

Health Mixes		
	Initial	Final
HM I VAR 4	21000	23000
HM II VAR 3	19500	21000
HM III VAR 3	19000	22500
HM IV VAR 2	18500	21000
HM V VAR 5	17100	20500

The bacterial count of fresh sample at dilution 10.3 ranged between 17100-21000 whereas in the three month old sample the count increased and ranged lower than the specified counts 30000-60000/g [12]. This shows that all the formulated food variations indicate that these variations retained desirable characteristics on storage for a period of three months. All the food formulations had a good shelf life and were found to be fit for consumption even after three months.

Among the best variations from each health mix, variation 5 of health mix V got the highest score of 16.6 in organoleptic evaluation because of its highest score in taste and colour, maximum nutrient content, minimum moisture content and peroxide value and with less total bacterial count. However based on the nutrient content and shelf life study, variation 5 of health mix V was adjudged to be the best and named as HEALTH PLUS and used for further nutrition intervention study. Health plus was formulated with ingredients namely wheat, soy flour, wheat germ, tomato, beetroot, sunflower seeds and jaggery in the ratio of 15:15:15:15:15:10:15. The fresh and powered immune boosters, modulators and regulators of health plus is given in Plates 8 and 9. Six variations formulated with variation 5 of health mix V is given in Plate 10.

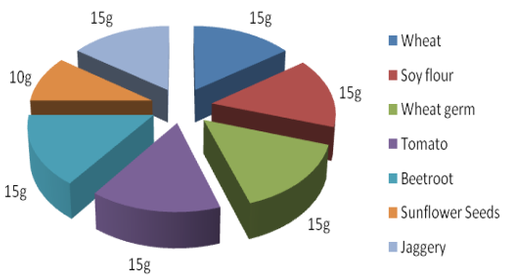

Figure 1. Proportion of health plus.

3.1.4 Nutrient Content of Standarised Health Plus

The nutrient content of standardized health plus is given in Tables 11.

Health plus was subjected to nutrient analysis using standard procedure. The moisture content of health plus was 9.9 g/100g of health mix. The energy content was 640 Kcal. Carbohydrate content was found to be 64.5 g and dietary fibre was 18.8 g in 100 g of health plus. Protein content was found to be 23.3g. Polyunsaturated fat content of the mix was 2.2 mg.

Table 11. Macronutrient content (per 100 g) of health plus

Macronutrients	Health Plus
Moisture (g)	9.9
Ash (g)	5.8
Energy(Kcal)	640
Carbohydrates (g)	64.5
Dietary fibre (g)	18.8
Protein (g)	23.3
Total fat (g)	4.3
Saturated fat (g)	0.6
Monounsaturated fat (mg)	0.6
Polyunsaturated fat (mg)	2.2

Table 12. Amino acids profile of health plus (per 100 g)

Amino acids	Health Plus	Amino acids	Health Plus
Histidine (mg)	430	Cystine (mg)	371
Isoleucine (mg)	486	Tyrosine (mg)	436
Lysine (mg)	600	Arginine (mg)	1087
Leucine (mg)	928	Alanine (mg)	765
Methionine (mg)	24	Aspartic acid (mg)	1130
Phenylalanine (mg)	595	Glutamic acid (mg)	2874
Threonine (mg)	500	Glycine (mg)	898
Tryptophan (mg)	282	Proline (mg)	882
Valine (mg)	726	Serine (mg)	684

Standardised health plus was assessed for its amino acid content using standard procedures. Hundred gram of health plus contains an appreciable amount of essential and non-essential amino acids. Histidine content of health plus was 430 mg and isoleucine content was 480 mg. Leucine content was 928 mgTryptophan content of health plus was 282 mg. Threonine content was 500 mg. Methionine content of health plus was very minimum around 24 mg. The Phenylalanine content was found to be 595 mg and valine content of health plus was 726mg A good ratio of non-essential amino acids which cannot be synthesised by the body was also present in

health plus. Health plus contained 3874mg of glutamicacid. The formulated health plus contains a good ratio of essential to non essential amino acids. Cystine content of health plus was 371 mg and tyrosine content was 436 mg. Health plus contained 1087 mg of arginine.

Aspartic acid content of health plus was 1130 mg and glutamic acid was 2874 mg. Glycine content of health plus was around 898mg. Proline content was found to be 882 mg. Serine content of health plus was 684 mg.

4. Conclusion

The findings of the study revealed that the ingredients namely wheat, soy flour, wheat germ, tomato, beet root, sun flower seed and jaggery which are available at our doorsteps in affordable cost by the layman are foods rich in immune boosters, modulators and regulatory to improve the immune status of the HIV positive women.

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