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DEVELOPMENT OF ZINC ENRICHED HEALTH DRINK MIX FOR CHILDREN

Sangeeta Pandey*, Affrin Noor

Department of Nutrition and Dietetics, Mount Carmel College,
Bangalore 560052, Karnataka, India.

ABSTRACT: Zinc deficiency is related to low immune system, increased episodes of diarrhoea and pneumonia in children. Zinc enhanced products has tremendous potential to improve children health. This study was conducted to develop zinc enriched health drink mix for children containing pumpkin seeds and watermelon seeds to boost immunity and enhance health and nutrition status among children. Two sets of health drink mix were formulated using pumpkin seeds (V1) and watermelon seeds (V2). Proximate calculations, biochemical analysis and sensory evaluation were studied. It was observed that variation 1(V1) of 30% incorporation of pumpkin seeds had highest protein (46.02g/100g), carbohydrates (67.25g/100 g) and energy content (483.09 kcal/100g) whereas, the highest content of zinc was found in variation 2 (V2) made of 30% incorporation of watermelon seeds (7.71 mg/100g). The highest content of fat and calcium was observed in variation 1 with 10% incorporation of pumpkin seeds and highest iron content was also observed in variation 1 with 20% incorporation of pumpkin seeds. Proximate analysis shows that the macronutrients and micronutrients values were significantly higher in the developed product. Hence the developed health drink mix can be used for zinc enrichment, which can reduce episodes of diarrhoea, pneumonia and increase immunity among the children.

Keywords: Zinc Deficiency, diarrhoea, immunity, pumpkin seeds, watermelon seeds.

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Corresponding Author: Dr. Sangeeta Pandey*

Department of Nutrition and Dietetics, Mount Carmel College, Bangalore 560052,
Karnataka, India. Email Address : pandey.sangeeta@yahoo.com

1. INTRODUCTION

Zinc is an important trace mineral that is needed to stay healthy. This element is second to iron in its concentration in the body. Zinc is vital for the normal growth and development of the reproductive organs and brain. It plays a role in the normal functioning of the immune system and many other processes in the body. Zinc deficiency is more common in the developing countries which leads to diarrhoea, pneumonia and malaria, mainly observed in infants. Zinc deficiency is common in children from developing countries due to lack of intake of animal foods, high dietary phytate content, inadequate food intake and increased fecal losses during diarrhoea. Routine zinc supplementation given to low birth weight babies for a year has resulted in substantial reduction in mortality [1]. Micronutrients deficiency is big challenge in children as consumption of fruits, vegetables and animal foods are very low among this group. There are many types of health drinks available hence, due to its easy consumption and acceptability by children it was most suitable food product to address the deficiency. The health drinks with mineral enrichments are required to bridge the gap between the requirement of the children and the common deficiencies, which sometimes proves to be fatal like diarrhoea and acute respiratory infection etc. Several studies have suggested that supplementation of zinc improves growth rate and reduces incidences of infections [2].

2. MATERIALS AND METHODS

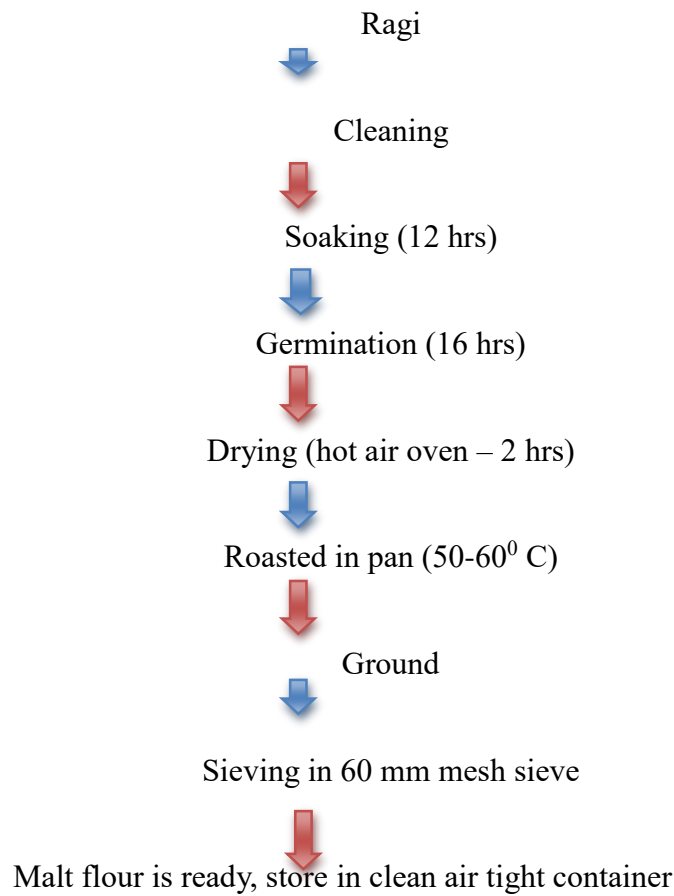
The study aimed to explore the potential use of different zinc rich food sources in terms of nutrient, color and sensory acceptance for children.

Formulation of health drink mix

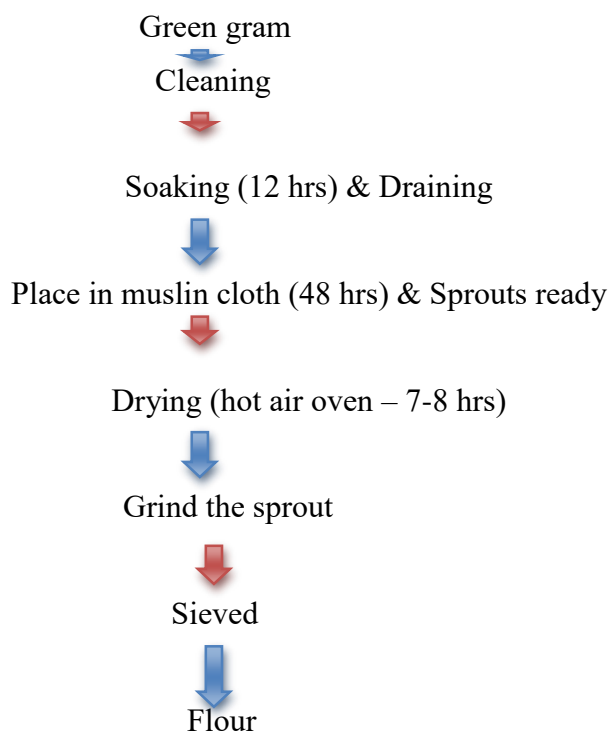
A Health drink mix was developed using malted ragi, sprouted green gram, skimmed milk powder, cocoa powder, sugar and zinc rich food sources such as dark chocolate, pumpkin seeds and watermelon seeds. In the formulation of health drink mix various zinc rich ingredients were added in different percentage. Ragi was procured from local market and was malted to improve the digestibility. Green gram was sprouted and ground to powder and sieved. Skimmed milk powder and cocoa powder was procured from the market in powder form. Pumpkin seeds and watermelon seeds were roasted, grounded and sieved. Processed ingredients were mixed in different proportions along with the zinc rich ingredients. Sugar was added to enhance the taste. Among zinc rich food sources, pumpkin seeds have 2.343 mg zinc in 30 % composition, 1.562 mg in 20% and 0.781 in 10%. Watermelon seeds have 3.33 mg zinc in 30%, 2 mg in 20% and 1 mg in 10%. Malted Ragi has gained importance because of its nutritional quality in terms of dietary fibre, functional fibre, starch pattern as well as high calcium and iron contents. Sprouted mixes can be used to develop snacks for children with nutritional and functional benefits [3,4]. Dark chocolate was included because it was rich in zinc and mostly liked by children.

Processing of Raw Materials

Processing of Ragi : Preparation of Ragi Malt

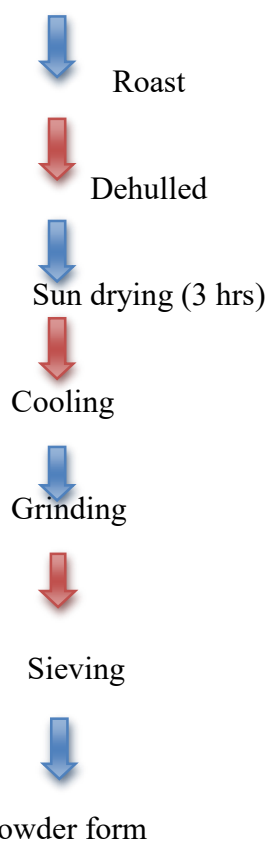


Processing of Green Gram



Processing of Pumpkin seeds and Watermelon seeds

Pumpkin seeds / watermelon seeds



Proximate/ chemical Analysis

For the Biochemical analysis the procedures used in the study are as follows. The control and variations were subjected to analysis of Protein, Moisture, Ash, Fat, Iron, Calcium and Zinc following standard protocols [5]. Computation of Energy and carbohydrate was done by the formula referred by AOAC [5]. Protein was estimated using Kjehdhal method. Digestion process was carried out where 0.2 gm of the sample was weighed into digestion tubes of the Gerhardt digester in triplets and one heaped spatula each of sodium and copper sulphate were added to each tube. 5 ml of concentrated sulphuric acid was also added and sample was digested at 300⁰ C - 1 hr and then 400⁰ C – 2 hr until the contents of the tubes were sky blue in colour. Further in distillation process 10 ml of each sample were transferred into the distillation tube of the automatic Gerhardt unit and 20 ml of the 2 percent boric acid to which was added 3-4 drops of the mixed indicator was placed in the collecting conical flask to trap the liberated ammonia. The unit was furnished with 40 percent NaoH and distilled water to facilitate operation. The titrated values obtained were incorporated in the equation to obtain the percent protein.

Moisture Estimation

An empty clean and dry container was weighed accurately. 5 gram of the sample was weighed accurately and put in the containers. Then the sample was dried in hot air oven at 110^o C overnight

and later was cooled in the desiccator. From the containers the moisture content was calculated.

Fat Estimation

Fat was estimated by Soxhlet's Apparatus. Five gram of sample was weighed into a thimble and closed with fat free cotton wool. The thimble was placed in the soxhlet apparatus attached to a pre-weighed flask and extracted for about 14-26 hours. Thereafter, the flask was retrieved from the apparatus with as little solvent in it as was possible. It was then transferred into an oven to evaporate the remaining solvent, leaving behind only the residue or extract. The flask was then cooled in desiccators after which it was weighed to estimate the fat .

Ash Estimation

The ash content of the sample was obtained by carbonizing dry powder over flame and ashing the samples completely by igniting the carbonized sample in muffle furnace. This was expressed as gram/ 100 g of the sample .

Mineral estimation (Iron and Calcium)

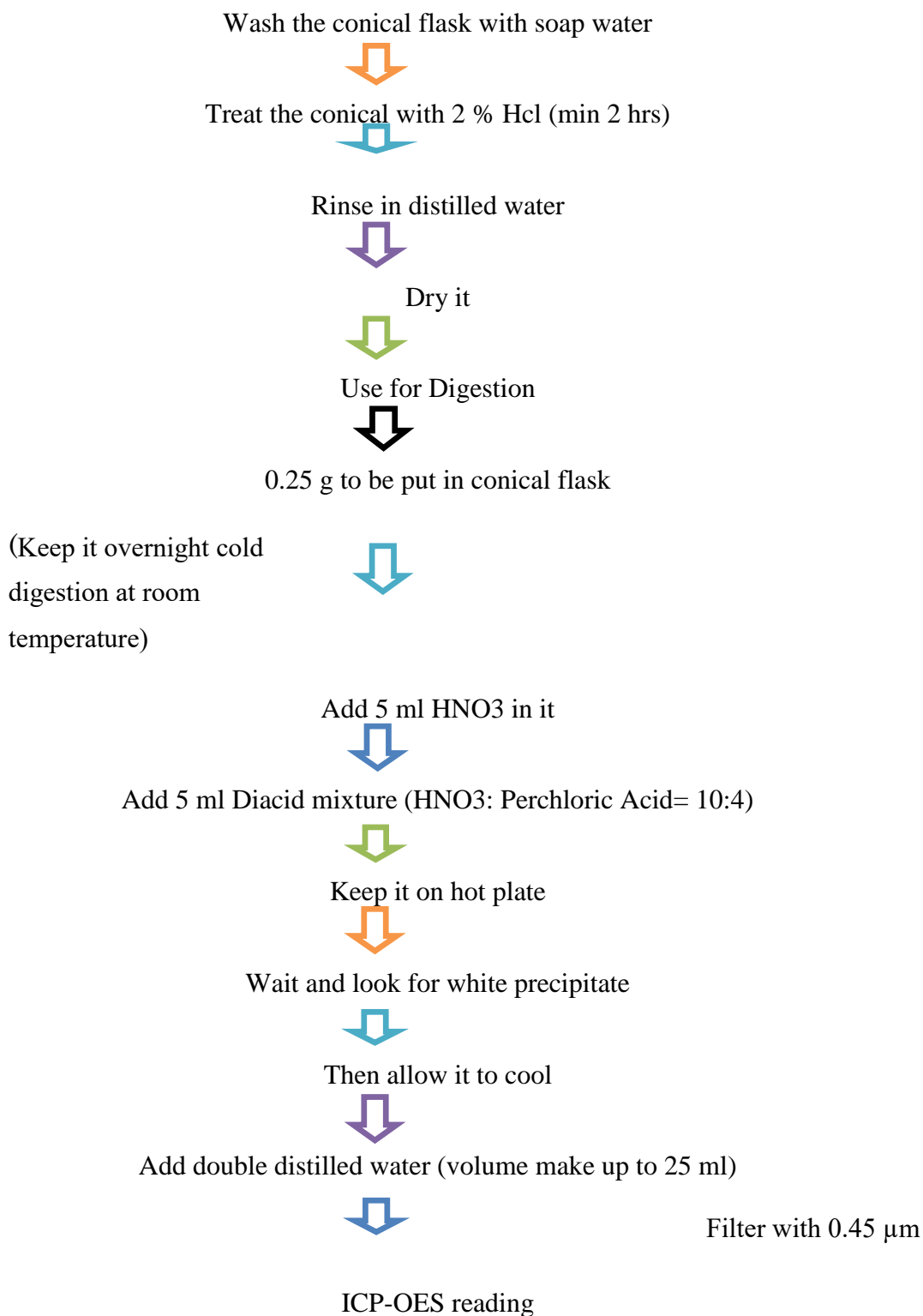
The mineral solution was prepared by dissolving the ash obtained from samples in muffle furnace using diluted hydrochloric (6N) acid.

Ashing of sample for preparation for mineral solution: Total ash in the sample was estimated by first weighing two grams of dry powder into a pre weighed crucible. For all samples, this was done in triplicates. Each crucible was placed on wire gauze and heated over a low flame until the material was completely charred and had stopped giving of smoke. The crucible was then transferred into a muffle furnace where the sample was heated to 550°C for three hours. It was then cooled in desiccators. At final stage, the ash was completely white in colour.

Preparation of mineral solution: To the ash that was obtained after burning followed by ashing was added 5 ml of a 1:1 solution of distilled water and fuming HCl. This mixture was then heated over a water bath to dryness before another 5 ml of the solution was added. It was heated further over the water bath until started fuming and at this point, the crucible was retrieved and its contents filtered into a 100 ml volumetric flask using Whatman No.4 filter paper. After thorough rinsing of the crucible and the filter paper, the volume was made up to the mark with distilled water. Aliquots of this mineral solution were taken for the estimation of all the other minerals such as Iron and Calcium.

Zinc Estimation

Zinc content was analysed by using Inductively Coupled Plasma- Optical Emission Spectrometer. (ICP-OES) [5].

Preparation of sample for zinc estimation by ICP-OES**ICP-OES Zinc Analysis Protocol**

Suitable dilutions i.e. 1:2:5 (4 ml sample: 10 ml water) were made from the extract with double distilled water so as to fit their absorbance with the range of standard curve. The diluted samples were fed to ICP-OES and the concentration was recorded in ppm using the standard curve. Three replications were maintained and their average was used for calculation of zinc content .

Sensory evaluation

Sensory evaluation was carried out using Quantitative Descriptive Analysis method. This method involves the evaluation of different sensory attributes based on a 9 point hedonic scale. The sensory evaluation was conducted by sensory panellists. The score card was developed incorporating 9 point hedonic scale ,code for the products and remarks by the panellists .

3. RESULTS AND DISCUSSION

A Health Drink Mix was formulated for children with zinc enhancement, which provides added benefit of nutrition for beneficial impact on the children health.

Table 1: Macro Nutrient Content of the health drink mix

Health Drink Mix		Macro Nutrient											
		Energy (kcal/100 g)			Protein (g/100 g)			Fat (g/100 g)			Carbohydrate (g/100 g)		
		Mean	± SD	t test	Mean	± SD	t test	Mean	± SD	t test	Mean	± SD	t test
Control		238.38	10.67	*	19.81	0.79	*	3.55	0.40	0.40	31.80	1.06	1.06
Variation 1	30%	483.09	8.22	37.45	46.02	0.13	67.52	14.87	1.95	1.95	67.25	1.88	1.88
	20%	435.33	24.80	15.07	40.04	0.34	48.46	14.30	4.45	4.45	61.54	5.20	5.20
	10%	373.42	13.24	16.40	31.46	0.52	25.45	15.97	2.59	2.59	55.07	2.86	2.86
Variation 2	30%	396.32	7.93	24.50	38.23	0.70	36.05	9.81	0.85	0.85	54.66	0.95	0.95
	20%	413.39	4.21	31.45	38.42	0.70	36.30	13.03	0.49	0.49	58.58	0.39	0.39
	10%	367.34	9.81	18.36	33.18	1.32	17.92	9.39	0.27	0.27	51.92	1.16	1.16
ANOVA – F		128.6			113.4			62.8			36.5		
Significance		Significance at 1%			Significance at 1%			Significance at 1%			Significance at 1%		
CD		51.2			12.3			3.6			8.4		

* t values were not calculated as it was single value

From table (1) it was observed that variation 1 of 30% incorporation had maximum energy and protein content. Fat content was maximum in variation 1 of 10% incorporation where as, carbohydrate content was found to be maximum in variation 1 with 30% incorporation. The energy, protein, carbohydrates, fat, iron, calcium and zinc were calculated and it was found that (table-2) the highest protein content of the health drink mix was in variation 2 of 20% incorporation (21.29 g), followed by variation 2 of 30% incorporation (20.35 g) and variation 1 of 20% incorporation (19.33 g) as compared to control (16.61 g). Legumes are very good source of protein, calorie, minerals and vitamins. In Nigeria, lima bean is consumed as cooked whole beans [6]. Maximum amount of fat was found in variation 2 of 30% incorporation (20.65 g), followed by variation 1 of 30% incorporation (19.03 g) and variation 2 of 20% incorporation (14.35g) whereas, control had

moderate amount of fat content (6.38 g). Nuts and seeds are rich in fat. Studies suggested that cocoa has been found to improve antioxidant status, reduce inflammation and helps to correlate with reduced heart disease risk [7]. Highest zinc content of the health drink mix was in variation 2. A high protein intake increases body fatness at 8 years of age, via an early adiposity rebound. A high fat low protein diet (such as human milk) is adapted to high-energy demand for growth in early childhood.

Table 2: Macronutrient and Zinc Content of the two selected Health Drink Mix Variations

Health drink mix		Protein (g/100 g)	Fat (g/100 g)	Zinc (mg/100 g)
Control		16.61	6.38	3.32
Variation 1	30%	17.41	19.03	4.28
	20%	19.33	13.27	4.3
	10%	16.71	9.36	4.09
Variation 2	30%	20.35	20.65	5.4
	20%	21.29	14.35	4.74
	10%	17.69	9.9	4.31

The maximum amount of zinc was in variation 2 of 30% incorporation (5.4 mg), followed by variation 2 of 20% (4.7 mg) and then in variation 2 of 10% (4.3 mg). Variation 1 also had high amounts of zinc content. In wound healing and tissue repair supplementation of zinc is beneficial only if zinc deficiency exists [8]. Table 3 depicts mean values and standard deviation of the control and variations. From the table it was found that zinc rich food sources used in the health drink mix were found to contribute significantly at 1% level. The highest iron content was in variation 1 of 20% incorporation. Calcium content was highest in variation 1 of 10% incorporation and lowest content in variation 2 of 30% incorporation. The maximum zinc content was in variation 2 of 30% incorporation and minimum in control. It was found to be significant (< 0.01). In accordance with the above table, the micronutrient contents of all variations were highly significant at 1%. Zinc deficiency has been reported to impair the development and functioning of cells that mediate both innate and acquired immunity. Hence zinc plays a role in improving immunity. In addition, zinc possibly also has anti-inflammatory properties [9]. Also it was found in the study that reduced risk of developing pneumonia in children were associated with supplementation of zinc [2].

Table 3: Micro Nutrient Content of the two selected Health Drink Mix Variations

Health Drink Mix		Micro Nutrient								
		Iron(mg/100 g)			Calcium (mg/100 g)			Zinc(mg/100 g)		
		Mean	± SD	t test	Mean	± SD	t test	Mean	± SD	t test
Control		7.64	0.02	*	30.69	0.06	*	3.57	0.01	0.01
Variation 1	30%	16.87	0.04	485.69	28.68	0.63	6.61	6.52	0.02	0.02
	20%	17.33	0.72	27.67	35.81	0.07	113.58	5.74	0.01	0.01
	10%	10.56	0.02	279.06	37.02	0.10	111.61	5.24	0.02	0.02
Variation 2	30%	7.98	0.02	28.13	24.88	0.03	183.83	7.71	0.05	0.05
	20%	9.52	0.02	167.98	27.78	0.04	83.13	5.41	0.06	0.06
	10%	9.84	0.02	210.41	28.81	0.05	48.97	3.90	0.01	0.01
ANOVA - F		31.8			9.21			11.4		
Significance		Significance at 1%			Significance at 1%			Significance at 1%		
CD		1.7			2.4			1.9		

* t values were not calculated as it was single value

Malting of ragi improves its digestibility, sensory and nutritional quality as well as it has pronounced effect in lowering the anti nutrients [10]. Malted Ragi is the best source of calcium for growing children and has many health benefits. In an observational study it was found that the prevalence of zinc deficiency was highest among children belonging to age group of 3-4 years and their mean serum zinc level was 62.42±23 mg/dL [11]. Table (4) depicts that in variation 1 of 30 % incorporation, maximum bacterial growth was observed (2.56±0.01), followed by variation 2 of 30 % incorporation (2.44±0.10) and least growth was also found in the same variation, which had 20% incorporation (2.04±0.06). The fungal growth was highest in variation 2 of 30 % incorporation (1.19±0.01), followed by variation 1 of 30 % incorporation (1.08±0.005) and least was found in control (1.04±0.04). The overall acceptability of control and variations was calculated and it was found that the control had maximum overall acceptability (3.55±6.82), followed by variation 1 of 30% incorporation (3.55±5.15) and least was variation 2 of 30% incorporation. The variation 2 of 30 % incorporation had maximum bacterial growth, moderate fungal growth and least overall acceptability (slightly disliked). Healthy drinks are important for growing children as it is easier for them to consume and it is nutrient dense which can fulfill the nutrient requirements of the children. Children tend to miss out or don't consume proper meal, which leads to malnutrition. Lack of dietary zinc in food leads to stunted physical growth, mental retardation, low immune system, diarrhea, pneumonia, and/or malaria. To prevent all these disease

conditions, zinc enhancement is important [12]. Ragi is a rich source of several phytochemicals, dietary fibre and minerals especially calcium [13,14]. Studies suggested that cocoa has been found to improve antioxidant status, reduce inflammation and helps to correlate with reduced heart disease risk [15]. The other benefits of the malting process include increased vitamin C content, phosphorus availability, and synthesis of lysine, tryptophan and calcium content [16]. Mild zinc deficiency may be widely spread even in the well-nourished population. Research shows that in population, which are deficient in zinc, supplementation or fortification would be helpful [17].

Table 4: Self life and overall acceptability of control and variations

Health Drink Mix	Zinc Variations (%)	Microbial Count		Overall Acceptability (Mean±SD)
		Bacterial (Mean±SD)	Fungal (Mean±SD)	
Control		1.33±0.06	1.04±0.04	3.55±6.82
Variation 1 Pumpkin seeds	30	2.56±0.01	1.08±0.005	3.55±5.15
	20	2.04±0.02	0.98±0.005	3.55±4.77
	10	1.95±0.06	0.91±0.05	3.55±4.79
Variation 2 Watermelon seeds	30	2.44±0.10	1.19±0.01	3.55±3.90
	20	2.04±0.06	1.03±0.02	3.55±4.24
	10	1.08±0.02	0.98±0.005	3.55±4.09

4. CONCLUSION

It can be concluded from the study that variation 1 of 30% incorporation was high in macronutrients with least bacterial and fungal growth. The ash content was high in variation 1 of 30% .The maximum overall acceptability was observed in variation 1 of 30% incorporation (like very much to slightly like). Hence Variation 1 (30% incorporation) was the best acceptable developed health drink mix. Zinc functions as an antioxidant and can stabilize membranes. This review explores aspects of zinc biology of the immune system and attempts to provide a biological basis for the altered host resistance to infections observed during zinc deficiency and supplementation [14, 18].Zinc was also found to have a therapeutic benefit in seven trials of acute diarrhea and five of persistent diarrhea [15,20].

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

There is no conflict of interest.

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