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Nutritional quality and Shelf life of Health Mix for Control of Anaemia among Adolescent Girls

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ABSTRACT
Nutritional anemia is one of India's most important public health problems. Adolescence is a susceptible period in the human life cycle for the growth of nutritional anemia. Anemia in adolescent girls contributes to maternal and foetal mortality and morbidity in future. 'Adolescence' as a phase between 10 and 19 years. Adolescence in girls has been distinguished as a special stage of transition from girlhood to womanhood. The overall health status is judged by the haemoglobin levels which found to be less than 11. With this background an attempt was made to develop a health mix for adolescent girls to control anemia. To develop a health mix for control of anemia and assess its nutritional quality and shelf life. It is an experimental research design to develop a health mix in two variations (V_1 and V_2) and select one product based on sensory evaluation scores rated by an expert panel on sensory evaluation cards. The health mix was evaluated for nutritional quality and shelf life before and after the storage at room temperature for three months from 5.4.2017 to 7.7.2017. The variation 1&2 of Health Mix was supplemented to Adolescent girls aged between 17 to 19 years, studying in Junior and Degree Colleges and having a Haemoglobin levels less than 10g%.The sample selected consisted of 70 adolescent girls who were willing to participate in the study. Among the sample selected 35 were included in the Control Group and 35 were included in the Experimental Group based on their willingness to take supplementation of Health Mix meant for alleviation of Anaemia and improving Haemoglobin levels. A lofty occurrence of anemia among adolescent females was established, which was higher in the lower socio-economic strata and amongst those whose parents were less educated. It was seen that anemia affects the overall nutritional status of adolescent females. Usage of Health mix and proper education will play an effective role in improving health of adolescents by increasing knowledge and changing their attitude.
Key words: *Anaemia, Haemoglobin, adolescent girls, health mixes, nutritional anemia.*

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INTRODUCTION

The world is domicile to 1.2 billion individuals aged 10-19 years. India has the prevalent population of adolescents (243 million) followed by China (207 million) and United States (44 million) [1]. Amongst adolescents, girls comprise a susceptible group, predominantly in developing countries where they are conventionally married at an premature age and rendered to a greater risk of reproductive morbidity and mortality [2]. In a family with restricted resource the female child is more prone to be ignored. She is deprived of good food and education and is exploited as an additional working hand to relay on the household works. The added burden of menstrual blood loss, normal or abnormal precipitates the anaemia too frequent. In India the prevalence of anaemia among adolescent girls is 90% [3]. Variations in prevalence rate of anaemia are seen within the country with the lowest prevalence of 33% being reported from Andhra Pradesh to highest of 98 % in

Rajasthan [4]. A high prevalence of anaemia in adolescent girls is a matter of great concern as they enter reproductive life soon after menarche [5]. Thus worldwide attention over iron deficiency anaemia in pregnancy has shifted recently from providing nutritional supplements during pregnancy to attempting to ensure that women especially adolescent girls have adequate iron stores prior to conception [6-13].

WHO report [17] reveals that anaemia prevalence in adolescent girls is very high ranging from 50% to > 90%. In 2006, the overall prevalence of anaemia has been reported to be extremely high at 90.1 % in adolescent girls of 11-18 years of age, from 16 districts in 4 regions of India. The study also confirms that 85 % of pregnant women were anaemic. The earlier study from Western India reports that in the low income group 80-90% had haemoglobin less than 12 %. In a study of adolescent girls (10-19 years) in urban slums of Southern India, Andhra Pradesh, anaemia prevalence is reported to be 67.9 % where moderate anaemia 37.05%, mild anaemia 21.42 % and 9.4 severe anaemia was seen, while another study from Ranga Reddy district of Andhra Pradesh reported anaemia prevalence in girls 13-15 years to be 83%.Where as under nutrition is reported (stunting) in one – third of adolescent population, prevalence of anaemia is almost universal. A similar high prevalence of anaemia in rural Rajasthan between 73.3% and 85.4% had been reported. About 62% of urban adolescent girls from the lower socio economic group are estimated to be anaemic. Anaemia in adolescent girls is now recognized to be a public health problem along with anaemia in other population groups such as young children and pregnant women [19-23].

Nutritional anaemia is one the India's major public health problems. Incidence of anaemia in school age children tends to increase with age and correspond with acceleration in growth during adolescence [15-16]. Globally, anaemia affects 1.62 billion people with about 69.4 million adolescents, 56 million pregnant women, and 468 million non-pregnant women estimated to be anaemic. Africa and Asia account for more than 85 per cent of the absolute anaemia burden in high-risk groups and India is the worst hit, where more than half of girls aged 15-19 are anaemic [18].There is a convincing evidence that iron deficiency and anaemia causes impaired growth, developmental delay, decrease physical activity, behavioural abnormalities, impairs cognitive function and school performance in adolescents. Iron deficiency in adolescent girls has serious implications on the future generation by adversely affecting the health of the foetus. Nearly one in every four adolescent girls aged 15-19 years in the developing world are married with South Asia the worst hit with 30 per cent of adolescent girls getting married at the age between 15-19 years [18].

Therefore our present study data provides an update with regard to nutrition and health status of anaemic girls and after the consumption of health mix with two variations, a drastic improvement of the health status in adolescent girls was quite clearly observed.

MATERIAL AND METHODS

Selection of Health Mix

For the present study the ingredients selected includes Ragi (*Eleusine coracana*), Wheat (*Triticum aestivum*), Rice flakes (*Oryza sativa*), Bengal gram (*Cicer arietinum*), Green gram whole (*Phaseolus aureus* Roxb), Black gram (*Phaseolus mungo* Roxb), Horse gram (*Dolichos biflorus*), Cowpea (*Vigna catjang*), Soya (*Glycine maxnerr*), and Sesame seeds (*Sesamum indium*) were procured from local market in Tirupati, Andhra Pradesh.

Formulation, Development and Standardization of Health Mix

The two Health Mix were formulated using the blending of multigrain such as cereals/ Cereal products, Millets, pulses/ legumes and oil seeds. All the ingredients were cleaned for dust and other extraneous materials and stored at room temperatures in an air tight container until further use. These Health mixes were developed with a purpose to provide nutritionally high biological value foods, proteins and concentrated source of energy along with micro nutrients and fibre. The Health mixes were developed using home level processing methods such as cleaning, washing, sprouting, drying, roasting to enhance the taste, flavour, acceptability and digestibility of nutrients and milling. All the ingredients were finely coarsely powdered and developed into Health mix with various combinations and proportions in the Department of Home science Laboratory.

A standard recipe is that which establishes procedures that will make possible production of high quality foods to be served for consumption.

For standardization two recipes with two variations each were formulated and served to the panel member's .The panel members evaluated the product according to the score card and gave remarks. Based on the remarks the recipes were modified and presented.

Composition of Health Mix

The Health Mix in two variations (V_1 and V_2) was developed to control Anaemia and promote general Health among the adolescent girls. Each variation consisting of 100g Health Mix provides two laddus (50g.laddu), the laddus were prepared with addition of Jaggery (10g) and Ghee (5g) to 100g of Health Mix. The Health Mix formulated does not contain Jaggery and Ghee, as it was subjected further investigation such as irradiation and shelf life. The combination and proportion of food materials used for each variation is as under Table 1.

Table 1: Composition of Health Mix

S.No	Food Materials	Health Mix	
		Variation -1	Variation -2
1.	Whole Wheat flour	20g	25g
2.	Rice flakes	25g	25g
3.	Sprouted Ragi flour	20g	25g
4.	Soya flour	5g	3g
5.	Roasted Bengal gram	5g	5g
6.	Cowpea	5g	3g
7.	Black gram	5g	3g
8.	Horse gram	5g	3g
9.	Green gram whole	5g	3g
10.	Gingelly seeds	5g	5g
	Total	**100g**	**100g**

The food materials/ingredients included in the recipe of Health Mix were procured weighed, cleaned, washed, sprouted, sun dried, roasted and powdered coarsely. The two laddus prepared with 100g of health mix, 10g of Jaggery and 5g ghee were subjected to organoleptic evaluation.

Sensory Evaluation

For sensory evaluation five point Hedonic scale test used to find out the overall acceptability of each sample and test scores were assigned for quality attributes like appearance, flavour, texture and taste and overall acceptability.

Preparation Score card for Sensory Evaluation

Hedonic scale was selected which is a preference test for testing the acceptability. Hedonic method is one where the judge expresses the degree of liking by checking a point on the scale ranging from Excellent to Poor. Separate column was given to write remarks.

The score card for sensory evaluation of products prepared with Health Mix was designed on the lines of criterion matrix to be evaluated on a five point scale as under;

Product code:						
S.No	**Sensory Evaluation attributes**	**Excellent 5**	**Very good 4**	**Good 3**	**Average 2**	**Poor 1**
1.	Appearance					
2.	Texture					
3.	Flavor					
4.	Taste					
5.	Overall acceptability					
6.	Remarks					

The panel members were oriented on the scoring method.

Acceptability of Developed Health Mix through Sensory Evaluation

The Sensory evaluation of Health Mixes were conducted in two stages, with variation 1 and 2 were prepared and subjected to sensory evaluation. The panel members were served coded products prepared Health Mix one after the other individually, along with a glass of water and sensory evaluation card. While serving the coded products for evaluation, care was taken to maintain a time gap of 15 minutes between service of each product, paper

napkins and hand each facilities was also provided, The products subjected to Sensory Evaluation included;

Table 2: Sensory Evaluation for the developed products before and after Irradiation

S.No	Health Mix and Variation	Type of product	No of Portions	Weight (g)	Code
1.	Non-Irradiated Health Mix-variation 1	Laddu	1	50g	E
2.	Health Mix- variation 1, Irradiated @ 0.25 kGy	Laddu	1	50g	F
3.	Health Mix- variation 1, Irradiated @0.75 kGy	Laddu	1	50g	G
4.	Health Mix- variation 2	Laddu	1	50g	H

Moisture Analysis by Oven Drying

An accurate assessment of moisture content in feed ingredients is important because moisture influences the nutritional evaluation of Health Mix .The oven-drying method for moisture determination has been widely used [24-27].

Ash Analysis

When organic contents of a material are burnt down, the left behind is ash. It is the inorganic content present in any material. If any material is heated to its burning point in presence of oxidizing agents, only ash is left behind. Any moisture content present is dried first and it would lead to sprinkling during heating. Fatty food samples leave moisture when dried which prevents spattering.

Carbohydrate Analysis by Difference method

Total carbohydrate by difference = total solids – protein – fat – ash. For high solids distilled spirits the total solids may include added sugar, citric acid, extracts, colors, and other Carbon/Hydrogen/Oxygen compounds. (OFFICIAL METHOD — SSD: TM:407) Official Methods of Analysis, 17th Edition, 2002; Horowitz; AOAC International, Maryland [27].

Protein Analysis of Health Mix by Kjeldhal method

The Kjeldhal method of nitrogen analysis is the worldwide standard for calculating the protein content in a wide variety of materials ranging from human and animal food, fertilizer, waste water and fossil fules.

Fat analysis by Soxhlet Fat extraction method

Fat plays an important role in many foods. Fat contribute to the flavour of food as well as it gives texture and also mouth feel to the food. It is an important component which gives us maximum energy. Approximately 9 Kcal energy per gram. Extra intake of fat mostly leads to obesity and below the level lead to malnutrition. It nourishes the body with all the essential fatty acid that body cannot synthesise and also help in building the body.

Iron Estimation by Wong's method

Iron is an absolute requirement for most forms of life, including humans and most bacterial species, Plants and animals as all use iron, and it can be found in a wide variety of food sources.

Calcium Estimation by Titrimetric method

One of the factors that establish the quality of a water supply is its degree of hardness. The hardness of water is defined in terms of its content of calcium and magnesium ions. Since an analysis does not distinguish between Ca^{2+} and Mg^{2+}, and since most hardness is caused by carbonate deposits in the earth, hardness is usually reported as total parts per million calcium carbonate by weight.

Thiamine Estimation

The determination of thiamine in widely different products has become a necessary as a result of the enrichment program, providing for a minimum and maximum vitamin content. However, the development of a completely suitable and accurate method for the determination of thiamine has proved a difficult task.

Determination of Riboflavin

Riboflavin is an essential micronutrient in the human diet. Because riboflavin is water soluble and not stored in appreciable amounts in the body, sources of riboflavin must be constantly consumed.

Dietary Fibre Analysis

The dietary fibre is edible parts of plants' carbohydrates that are resistant to digestion in human small intestine. Diets naturally rich in dietary fibre support to prevent constipation, improve gastrointestinal health, glucose tolerance and the insulin response, and reduce the risk of colon cancer, hyperlipidemia, hypertension and other coronary heart disease risk factors.

Statistical Analysis

All statistical analysis was done in triplicate and average values are calculated. Data were presented as mean ± Standard Deviation. The results were statistically analysed by one way analysis of variance and means were compared using bonferroni post hoc test with least significant differences procedure at 0.05 levels were used to describe the significance of differences between control and irradiated samples. Graph pad prism 3.1 version was used as statistical analysis software.

RESULTS AND DISCUSSION

Nutritional Supplementation of Health Mix to Adolescent girls.

The variation 1 of Health Mix was supplemented to Adolescent girls aged between 17 to 19 years, studying in Junior and Degree Colleges and having a Haemoglobin levels less than 10g%

The sample selected consisted of 70 adolescent girls who were willing to participate in the study. Among the sample selected 35 were included in the Control Group and 35 were included in the Experimental Group based on their willingness to take supplementation of Health Mix meant for alleviation of Anaemia and improving Haemoglobin levels.

Sensory Evaluation of Health Mix

The Health Mix developed to improve the haemoglobin levels of adolescent girls (17 to 19 years), whole Haemoglobin levels were less than 10g%. The composition of the Health Mix with two variants; V1 and V2 is shown in Table 3.

Table 3: The Food Composition of the Health Mix

S.No	Food Materials	Health Mix	
		Variation 1	Variation 2
1.	Whole wheat flour	20g	25g
2.	Rice flakes	25g	25g
3.	Sprouted ragi	20g	25g
4.	Soya flour	5g	3g
5.	Roasted Bengal gram dhal	5g	3g
6.	Cow pea	5g	3g
7.	Black gram dhal	5g	3g
8.	Horse gram dhal	5g	3g
9.	Green gram	5g	3g
10.	Gingely seeds	5g	5g
Total		100	100

Table 3 depicts the nutrient composition of Health mix. The Variations of Health Mix consisted of cereals and millets(65%), pulses(35%) and oil seeds(5%), that is 65:30:5 ratio whereas the Variation 2 consisted of cereals and millets(75%), pulses(20%) and oil seeds(5%), that is 75:20:5 ratio respectively.

The nutrient composition of the Health Mix was calculated using the nutritive value of Indian foods (ICMR, 2011) and presented in Table 4.

Table 4: The Nutrient Composition of the Health Mix

S.No	Nutrients	Variation1	Variation 2
1	Moisture(g)	11.725	11.884
2	Protein (g)	14.052	12.228
3	Fat(g)	4.187	4.425
4	Carbohydrates (g)	65.033	66.312
5	Energy (Kcals)	348.16	355.3
6	Dietary fibre(g)	2.328	2.245
7	Calcium(mg)	207.09	211.07
8	Iron(Mg)	9.9405	9.1301
9	Vitamin A(µg)	61.45	60.79
10	Thiamine (mg)	0.3809	0.4206
11	Riboflavin(mg)	0.1722	0.1696

The Table 4 shows that the nutrient composition of the two variations (V1 and V2). From the table it was clear the Health Mix did not differ much in their nutrient composition. The protein and energy content of Variation2 was slightly more than the Variation 1. Thus the nutrient composition of both the Variations Health Mix was almost similar.

The Dasa Dhanya Mix (10 ingredients) was prepared followed by standard procedures cleaning, washing, sprouting (ragi and green gram only) sun drying for three days and powdered coarsely with both the variations a and 2 of Health Mix laddus (balls) were prepared by adding Jaggery (10g) and ghee(5g) to 100g of Health Mix . The laddus prepared with two variations (V1 and V2) of Health Mix were subjected to sensory evaluation. Ten panel members selected for the study evaluated the products on a 5 point hedonic scale for 5 attributes: appearance, texture, taste, flavour, overall acceptability. The mean scores of each attribute was calculated and presented in table 5.

Sensory Evaluation of Health Mix before irradiation

Table 5: Mean Sensory Valuation Sores of Two Variation

S.No	Sensory evaluation attributes	Mean Score of Non Irradiated Health Mix	
		Variation 1	Variation2
1	Appearance	4.2	4.2
2	Colour	4.2	4.3
3	Taste	4.3	4.2
4	Flavour	4.4	4.1
5	Overall acceptability	4.3	4.2

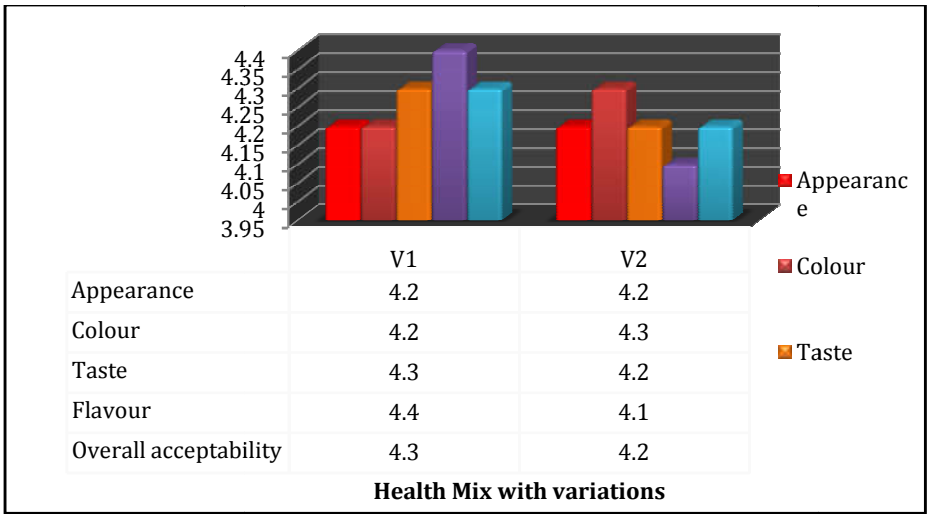


Table 5 and fig-1shows the mean sensory evaluation scores of two variations: V1 and V2 of Health Mix. From the data it was clear that the variation one had slightly higher scores for all the attributes than the variation 2. The means scores for both the variations were

between 4 to 5 indicating that both the products were very good. Based on the sensory evaluations means scores the variation1 of Health Mix was selected for nutritional quality assessment and nutritional intervention to adolescent girls.

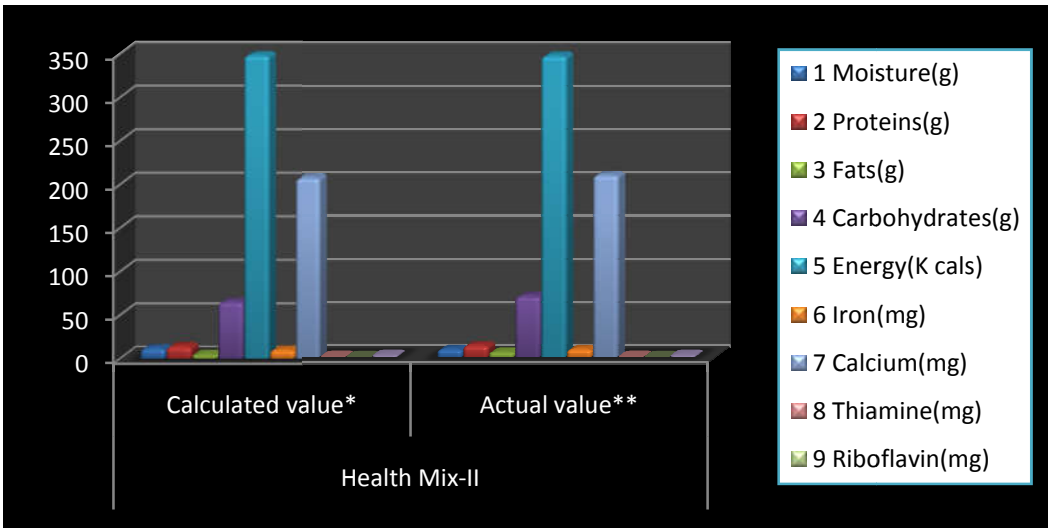
The calculated and chemically analysed Nutrient composition of Health Mix

The nutritional quality of Health Mix before irradiation was calculated using the Nutritive Value of Indian Foods (ICMR 2011) and also analysed standard procedures and presented in table 6 and Figure 1.

Table 6: Comparison of Nutritive value of Health Mix

S.No	Nutrients	Health Mix	
		Calculated value*	Actual value**
1.	Moisture(g)	11.725	9.10
2.	Proteins(g)	14.052	13.90
3.	Fats(g)	4.187	5.46
4.	Carbohydrates(g)	65.033	71.54
5.	Energy(K cals)	348.16	348.16
6.	Iron(mg)	9.9405	9.10
7.	Calcium(mg)	207.09	210
8.	Thiamine(mg)	0.408	0.42
9.	Riboflavin(mg)	0.1722	0.1722
10.	Fibre(g)	2.328	2.28

* As calculated using nutritive value of Indian foods (ICMR, 2010)
**As analysed using standard procedures [27]



The table 6 and figure 2 shows that there was not much difference in the values of nutrients (calculated manually and chemically analysed) of samples of Health mix.

Difference in Moisture, Ash and Protein content Health Mix

Moisture plays a key role in food irradiation. Water acts as a medium for the free radicals to move and interact with other food components. Free water is thus important in promoting the secondary effects of irradiation. This fact has been proven when foods are irradiated in frozen state with the production of limited secondary effects. Secondary effects are also very limited if the moisture content is less than 12%.

Table 7: Mean Moisture, Ash and Protein content Health Mix

S.No	Health Mix	Nutrient	Source of Variation	Sum of squares	df	Mean square	f-value
1	**HM	Moisture	Between groups	17.898	2	8.949	5593.2
			Within groups	0.009600	6	0.001600	
			Total	17.908	8		
2	HM	Ash	Between groups	0.9824	2	0.4912	359.41
			Within groups	0.008200	6	0.001367	
			Total	0.9906	8		
3	HM	Protein	Between groups	0.3224	2	0.1612	117.95
			Within groups	0.008200	6	0.001367	
			Total	0.3306	8		

Difference in Fat, Carbohydrate and Fibre content of Health Mix

Table 8: Difference in Fat, Carbohydrate and Fibre content of Health Mix

S No	Health Mix	Nutrient	Source of Variation	Sum of squares	df	Mean square	f-value
1	HM	Fat	Between groups	3.361	2	1.681	1229.8
			Within groups	0.008200	6	0.001367	
			Total	3.370	8		
2	HM	Carbohydrate	Between groups	27.983	2	13.992	10238
			Within groups	0.008200	6	0.001367	
			Total	27.992	8		
3	HM	Fibre	Between groups	0.05180	2	0.02590	18.951
			Within groups	0.008200	6	0.001367	
			Total	0.06000	8		

HM -indicates Health Mix

Table8 and indicates that there was no difference either between groups and within groups of samples of Health Mix in Fat, Carbohydrate and Fibre content , as the F-value was greater than 0.001.

Difference in Iron, Calcium, Thiamine and Riboflavin content Health Mix

Table 9: Mean Iron, Calcium, Thiamine and Riboflavin content Health Mix

S No	Health Mix	Nutrient	Source of Variation	Sum of squares	df	Mean square	f-value
1	HM	Iron	Between groups	48.716	2	24.358	17823
			Within groups	0.008200	6	0.001367	
			Total	48.724	8		
2	HM	Calcium	Between groups	254.34	2	127.17	93051
			Within groups	0.008200	6	0.001367	
			Total	254.35	8		
3	HM	Thiamine	Between groups	0.2966	2	0.1483	108.51
			Within groups	0.008200	6	0.001367	
			Total	0.3048	8		
4	HM	Riboflavin	Between groups	0.2467	2	0.01233	9.024
			Within groups	0.008200	6	0.001367	
			Total	0.03287	8		

The table 9 indicates that there was no difference either between groups and within groups of samples of Health Mix in Iron, Calcium, Thiamine and Riboflavin content as the F-value was < 0.001.

Table 10: Mean Nutrient content of Health mix

S No	Nutrient	Sample	Non Irradiated	F-Value	Significance
	Moisture	Health mix	11.725±0.03	5593.2	0.0001
	Ash	Health mix	2.52±0.03	359.41	0.0001
	Protein	Health mix	13.90±0.03	117.95	0.0001
	Fat	Health mix	5.46±0.03	1229.8	0.0001
	Carbohydrate	Health mix	71.54±0.03	10238	0.0001
	Fibre	Health mix	2.28±0.03	18.951	0.002
	Iron	Health mix	9.10±0.03	17823	0.0001
	Calcium	Health mix	210±0.03	93051	0.0001
	Thiamine	Health mix	0.42±0.03	108.51	0.0001
	Riboflavin	Health mix	0.225±0.03	108.51	0.0155

Shelf life of Health Mix

The Shelf life of Health Mix ((Variation 1) and Health Mix of (Variation 2) was assessed by microbiological analysis, pH and rancidity. The microbial growth in the Fresh Health mixes and preserved (for 15 to 120 days) Health Mixes were examined for number of colonies formed on the Agar plate. The Non- Irradiated sample that is the fresh sample at 24hrs exposure resulted in $4x10^3$ CFU/g but in 15 days, 30 days & 120 days old sample the CFU/g was shown in table 11.

The microbiological analysis was expressed in terms of Colony Forming Units per gram and the CFU/g of all the samples were compared. The more the CFU/g, the lower the shelf life of the health mix.

Table-11: Microbiological analysis of Health Mix

S.No	Sample	24hrs	48hrs
1.	Fresh sample	$4x10^3$	$6x10^3$
2.	15 days old	$8x10^3$	$1.8x10^3$
3.	30 days old	$25x10^3$	$0.9x10^3$
4.	120 days old	$36x10^3$	$0.4x10^3$

The results of Micro biological analysis indicates that the lower CFU/g of the sample, was present in Non- Irradiated samples of Health Mix. Which reveals that they were effective in improving the shelf life of the Health mix.

CONCLUSION

The Present study on "Nutritional quality and Shelf life of Health Mix for Control of Anaemia among adolescent girls" is an experimental research conducted in seven phases. Besides experimental design, included to study the impact of non irradiated Health mix developed for adolescent girls suffering from anemia. The present study was undertaken to develop the Health Mix in two variations (V_1 and V_2) in order to control Anaemia and promote general Health awareness among the adolescent girls.

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