

Development of Millet Based Moringa - Amla Snack Bar for Iron Deficiency in Adolescents

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Recibido: 28/febrero/2024. Aceptado: 13/mayo/2024.

ABSTRACT

Iron intake is crucial for promoting adolescent health as their growth needs deplete the body's iron stores. However, junk eating by adolescents leads to inadequate dietary intake. Research studies have shown that incorporating low-cost, non-heme iron-rich moringa leaves into food products, may help meet daily iron needs. Although moringa leaves are a part of everyday diet in South Indian households, underuse is also observed.

Aim: To develop a millet-based snack bar, incorporated with dried moringa leaf flour and Amla powder.

Methods: Moringa Leaves were procured from local farm. Snack Bar was prepared using Moringa Flour at different concentrations (20%,40% and 60%) and standardized by mixing it with groundnut flour, toasted sesame seeds, Ragi and Kambu millets, dried amla powder ghee, and Jaggery. The sensory evaluation was done using 30 semi trained panel members on a 5 point hedonic rating scale and the accepted product was nutritionally analyzed.

Results: The Moringa Amla Millet Bar with a 20% incorporation showed better overall acceptability, when compared to Moringa Amla Millet Bar with 40% and 60% variations. There was a significant difference at 1% level among 20%, 40%, and 60% variations. The 20% level incorporation had a high product acceptability index of 88% compared to the 40% and 60% levels. The nutrient analysis of the accepted indicated that the following values per 100gm. 57.5mg of Iron,11.8 mg of vitamin C, 12.1g of Fiber, 12.2 mg of Protein, 345mg of cal-

cium, 19.4mg of Zinc, and provides a substantial energy source of Kcal.

Conclusion: The nutritious Moringa leaves with surplus health benefits shall be well utilized by incorporating it in various nutritious recipe preparation that may serve as iron supplements for growing adolescents.

KEYWORDS

Moringa Olifera, Iron deficiency, Functional Food, Adolescent, Anemia.

INTRODUCTION

Iron deficiency anaemia is prevalent nutritional disorder affecting adolescents worldwide. As adolescent's iron stores tend to deplete during the growing process, a balance intake of iron, a micro nutrient is crucial¹. Factors such as poor dietary choices and peer influences often lead to insufficient intake among them². This issue remains a significant concern in low-income group countries like India, despite extensive iron supplementation programs targeting adolescents. Recently Moringa, which is rich in iron has gained popularity as a sustainable resource³. This plant has been a staple in Indian households for centuries. Its drought resistance requires minimal agricultural care enabling global cultivation. Research has identified thirteen moringa species from the "Moringaceae" family, native to Africa, Asia, islands in the Pacific and the Caribbean as well as South America. Of these M. Peregrina, M. Stenopetala and M. Olifera are the three species that are cultivated on a large scale in India, the Philippines and Pakistan⁴.

Moringa leaves, a viable source of energy and micronutrients including iron, may provide an ideal solution to meet the nutrient requirements of the growing adolescents⁵. Recent studies have shown that anaemia indicators improve

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with consumption of Moringa leaves due to its high iron content. This has led to an increase of development of food products incorporating Moringa leaves such as, cookies, jam, energy drinks⁶. These products could cater to the iron needs of adolescents who prefer snacking over meals. However the acceptability of Moringa incorporated snacks varies depending on the proportion of Moringa leaf flour to the base materials⁷. A minimum of 4 % concentration in snack development has shown to increase haemoglobin levels in anaemic adolescent girls⁸. In contrast, an animal study revealed that the bioavailability of iron from moringa is low⁹. However, an invitro study advocated for a sustainable food based approach of combining ascorbic acid with Moringa to increase bioavailability of iron¹⁰.

The use of Vitamin C capsules with Moringa Leaves resulted in an increase of 3.1g% of haemoglobin compared to 1g% increase without vitamin C. This emphasizes the importance of a sustainable food-based approach, such as adding vitamin C or ascorbic acid. Indian gooseberry or Amla, known for treating Anemia is a rich source of ascorbic acid, with 170.11mg/100g in its sun dried powder form¹¹. Insights from previous research on the development of snack bars shows the use of rice flour, millet flour, ground nut flour, wheat flour, and oat flour¹²⁻¹⁴. A large body of evidence suggests that using groundnut flour as a base for the production of snacks is helpful for body building and supplies adequate iron and are consumed as snack bars "chikkies"^{15,16}. Despite their proven nutraceutical properties and long history as staple foods, millets have struggled to gain popularity among adolescents, particularly as a snack preference¹⁷. Interestingly research evidences demonstrated the beneficial effects of millets in improving iron deficiency Anemia¹⁸. This study proposes the development of millet- peanut based snack bar, enriched with bioavailable iron, and designed to be appealing to adolescents. This could serve as a sustainable resource for meeting their iron requirements. It will incorporate Moringa Leaf Flour, known for its rich iron content, and Amla flour. The present study aims to analyze and evaluate the nutrient content of Moringa Amla Millet Snack bar.

MATERIALS AND METHODS

The raw ingredients used for the production of the Iron Rich (Plant-Based Moringa Amla) Millet Snack Bars are pearl millet and finger millet flakes, groundnuts, Moringa Olifera leaf powder, dried amla powder, sesame seeds, jaggery, and ghee.

Processing of Moringa Leaf Powder

Moringa leaf powder is made from fresh moringa leaves that are carefully selected from a nearby farm (Ayapakkam). Then, the leaves were de-stalked, and leaf petals were carefully removed by hand, washed with water, and only those that were free from blemishes were chosen for drying. The

drying process takes place in a shaded area for 3-5 days to preserve the colour and nutrients. After complete drying, the leaves are ground to obtain Moringa leaf flour using a mixer grinder. The unsieved flour is stored in a container in the refrigerator.

Processing of Amla Powder

To prepare the Amla powder, the amla purchased from local market is first thoroughly washed with water. Then the fresh and healthy ones, free from any blemishes, infection, or damage are separated and cut in to small pieces. These pieces are then sun dried for 3-5 days and ground into fine using mixer grinder. The amla powder is stored in an air tight container in the refrigerator.

Processing of Millets

Ragi and kambu (finger millet and pearl millet) are rich in iron and inexpensive staple foods in South Indian regions. The millets are soaked overnight, rolled under pressure using traditional methods, and dried in the sun. They are fluffed up at 120 degrees Celsius for 5 to 10 minutes and then ground into coarse flour. Ragi processed by soaking and flattening that result in ragi flakes is a rich source of iron (5.9%), compared to the normal grain form (3.9%).

Processing of Ground Nuts

Roasted ground nuts are processed by heating the nuts up to 180 °C for around 12–15 min or at 160 °C for 40–60 min, depending on the moisture content. The roasted groundnuts are then cooled and ground into coarse flour. It has an iron content of 4.6 g, a low glycemic index, a high protein content and dietary fibers, is rich in antioxidants, and has greater anti-cancer properties. The soaking process helps to remove phytates and oxalates that inhibit iron absorption.

Procedure for Preparation of Bars

All the ingredients used in this recipe are weighed beforehand. The flour made from coarsely ground millet and finger millet (Ragi, Kambu) flakes, roasted crushed ground nuts, roasted sesame seeds, Moringa leaf powder, and Amla powder is thoroughly mixed until a uniform mixture is obtained. Ghee is then added to this mixture. In the meantime, jaggery is melted with water, filtered, and cooked until it reaches a ball consistency upon dripping into cold water. The jaggery syrup is then added to a blender containing a uniform mixture to form a thick, consistent, moistened mixture. While the mixture is still warm, it is spread on a greased tray, and the bars are cut and set to room temperature. Three blends of moringa flour in proportion to ground nut powder were used, namely 20 mg (M_3), 10 mg (M_2), and 4 mg (M_1), in preparing the bar.

Table 1. Compositions for variations of Moringa Amla Millet Bar (100gm)

S. No.	Ingredients	Sample (M ₁)	Sample (M ₂)	Sample (M ₃)
1	Dried Amla Powder	1 gm	1 gm	1 gm
2	Dried Moringa Leaf Powder	4 gm	6 gm	10 gm
3	Sesame Seeds	5 gm	5 gm	5 gm
4	Ragi Flakes	15 gm	15 gm	15 gm
5	Kambu Flakes	15 gm	15 gm	15 gm
6	Ground Nut	20 gm	18 gm	14 gm
7	Ghee	5 gm	5 gm	5 gm
8	Jaggery	35 gm	35 gm	35 gm

Sensory Evaluation

Sensory evaluation for three Moringa Amla Millet Bar variants was assessed using a 5-point hedonic scale with thirty semi-trained panellists. Appearance, aroma, taste, texture, and sweetness were the five parameters evaluated on a scale of 1 to 5, with the responses ranging from dislike very much to like very much. The product acceptability index (PAI%) was calculated using the formula:

$$\text{PAI}\% = \frac{(\text{Average grade obtained for a product} \times 100)}{(\text{Maximum grade given to a product})}$$

Nutrient analysis of best acceptable Bar

The physio-chemical parameters analysed included moisture (dried at 105 °C) (AACC, 2010), fat (Soxhlet apparatus), total protein (Kjedldhal methods, Nx6.25), and the AOAC (1990) method. The total energy value of each bar formula was estimated using the following conversion: 4 kcal = 1 g of protein, 4 kcal = 1 g of carbohydrates, and 9 kcal = 1 g of fat¹⁹.

Phytochemical Screening of Moringa Amla Millet Bar

GC Program

The given sample was extracted with methanol and analyzed through Gas Chromatography – Mass Spectrometry/Mass Spectrometry for identification of different compounds²¹. The Gas Chromatography (GC) program utilized the Rtx-5MS column, comprising 5% Diphenyl and 95% Dimethyl polysiloxane, with dimensions of 30 meters length, 0.25mm inner diameter, and 0.25µm film thickness. The equipment employed was the TSQ 9000 Triple Quadrupole GC-MS/MS system, featuring a carrier gas flow rate of 1ml per minute with a split ratio of 10:1. Detection was per-

formed using the TQ Quadrupole Mass Spectrometer, controlled by the Xcaliber software. Each sample was injected at a volume of 1µl. The oven temperature program initiated at 110°C, maintaining this temperature for 3.50 minutes before ramping up to 200°C at a rate of 10°C per minute with no hold time. Subsequently, the temperature was further increased to 280°C at a rate of 5°C per minute, maintaining this temperature for 12 minutes. The injector temperature was set to 280°C throughout the analysis. The total running time for the GC method was 40.50 minutes, facilitating efficient separation and analysis of the target compounds. This comprehensive GC program ensured precise and reliable detection of analytes within the specified parameters, thereby enabling accurate analysis and interpretation of the sample constituents.

MS Programme

The analysis was conducted using the NIST Version-2011 library in conjunction with the Gas Chromatography-Mass Spectrometry (GC-MS) system (Figure 1). The inlet line temperature was maintained at 290°C, while the source temperature was set to 250°C. Electron energy of 70 electron volts (eV) was applied during ionization for efficient fragmentation of analytes. Mass scanning was performed within the range of 50 to 500 atomic mass units (amu), allowing for comprehensive detection of target compounds. A solvent delay of 0 to 3.5 minutes was implemented to ensure proper separation and elution of analytes. The total running time for the Mass Spectrometry (MS) analysis was 40.50 minutes, providing sufficient duration for the accurate identification and quantification of sample constituents. This combination of parameters and settings facilitated robust data acquisition and analysis, enabling the characterization of compounds present in the sample with high precision and reliability.

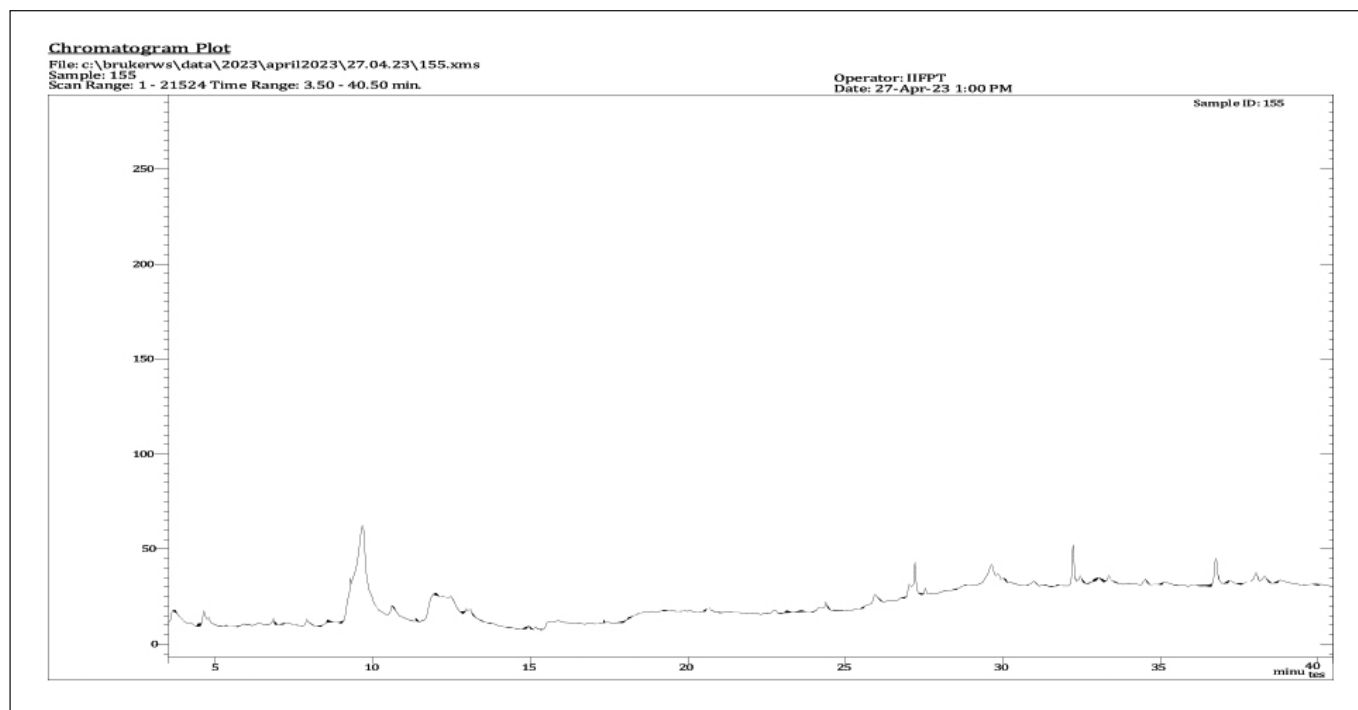


Figure 1. Phytochemical screening by MS Program

RESULTS

Sensory Evaluation

The product acceptability index for the product showed a significant improvement, with the 20% variant reaching an impressive score of 87%. This is a notable improvement when compared to the acceptability index of 84% for the 40% variant and 82% for the 60% variant.

The scores of sensory attributes in Table 2 state that in the three variants, the 20% Moringa Amla Millet Bar showed higher scores for all sensory characteristics, which included appearance (4.5 ± 0.63), texture (4.8 ± 1.06), sweetness (4.7 ± 0.62), aroma (4.4 ± 0.83), flavour (4.5 ± 0.73), and taste (4.7 ± 0.77).

The results indicated in Table 3 that there was a significant difference at a 1% level among 20%, 40%, and 60% variations. The comparison of overall mean sensory scores between the three samples (M_1 , M_2 , and M_3) was conducted using a one-way ANOVA (F value).

The P value of 0.0001** indicates that the group mean values are significantly different from each other. Among the three samples, the M_1 variant has the highest overall mean sensory score of 27.7 ± 1.41 and is considered the most acceptable. However, the M_2 and M_3 samples, with mean scores slightly lower but still close to M_1 , are also deemed acceptable at a moderate level, meaning they met these standards to a lesser extent.

Table 2. The mean score of sensory attributes

S. No.	Category	Mean Sensory attributes		
		M_1	M_2	M_3
1	Appearance	4.5 ± 0.63	4.3 ± 0.621	4.4 ± 0.596
2	Aroma	4.4 ± 0.83	4.2 ± 0.702	4.3 ± 0.834
3	Taste	4.7 ± 0.77	4.1 ± 0.834	4.2 ± 0.681
4	Sweetness	4.7 ± 0.62	4.0 ± 1.031	3.8 ± 0.788
5	Texture	4.8 ± 1.06	4.5 ± 0.894	4.4 ± 0.730
6	Flavor	4.5 ± 0.731	4.3 ± 0.937	4.4 ± 0.691

Table 3. Comparison between the overall mean sensory scores of three samples (One way ANOVA)

S. No.	Sample	N	Mean & SD	F Value	P value
1	M ₁	30	27.7±1.41	7.784	0.001**
2	M ₂	30	25.2±3.11		
3	M ₃	30	24.9±3.90		

** - Significant at 1% level.

The table 4 shows that sample M₁ is significantly different from both sample M₂ and sample M₃. Additionally, the mean value of sample M₁ is higher than the mean values of both sample M₂ and sample M₃. Therefore, we can conclude that sample M₁ is better than samples M₂ and M₃ in terms of sensory attributes. According to the 5-point rating scale, sample M₁ was found to be most acceptable in terms of appearance, with a mean score of 4.5. Its texture was also rated as the most palatable by several panelists, with a high mean score of 4.8. Considering the mean score of 4.7 for sweetness, M₁ appears to be the appropriate sample. There were no noticeable differences noted in the aroma, flavour, and appearance characteristics of the samples. The aroma, flavor, and appearance characteristics of the samples differ significantly.

Nutrient Analysis of the best acceptable Moringa Amla Millet Bar

The nutrient composition of the M₁ Moringa Amla Millet Bar revealed that it was calorically denser (375 kcal) with a minimal moisture content of 5%. The total ash content was 2.37%. Total iron content as Fe was 57.1 mg, vitamin C content was 11.87 mg, and zinc content was 19.2 mg. The protein composition was 12.2, and the bar was also rich in dietary fibre (9.8 mg), as represented in Table 5.

Table 5. Nutritional Analysis of 100g of Moringa Amla Millet Bar

S. No.	PARAMETERS	RESULTS
1	Energy (By Calculation)	375
2	Carbohydrates (By difference)	73.9
4	Protein (Nx 6.25)	12.23
5	Dietary Fiber	9.8
6	Total Sugar	42.0
	Added Sugar as Sucrose	33.4
7	Sodium as Na	69.2
8	Calcium as Ca	202
9	Magnesium as Mg	147
10	Zinc as Zn	19.2
11	Iron as Fe	57.54
12	Vitamin C	11.87

Table 4. Games – Howell multiple comparison test

S. No.	Sample (I)	Sample (J)	Mean difference (I - J)	SE	P value
1	M ₁	M ₂	2.500	0.624	0.001**
		M ₃	0.257	0.758	0.824
2	M ₂	M ₁	-2.500	0.624	0.001**
		M ₃	0.267	0.911	0.954
3	M ₃	M ₁	-2.767	0.758	0.002**
		M ₂	-0.267	0.911	0.954

** - Significant at 1% level. * - Significant at 5% level.

Total sugar amounts to 42.0 grams, with 33.4 grams attributed to added sugar, specifically sucrose. Sodium content is measured at 69.2 milligrams as sodium. Additionally, it provides 202 milligrams of calcium, 147 milligrams of magnesium, milligrams per serving.

Compounds identified in the methanolic extract of Moringa Amla Millet Bar by GC & MS Program

The compounds listed exhibit diverse chemical structures and molecular compositions. These include 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- with a molecular formula of C₆H₈O₄ and a molecular weight of 144, present at a concentration of 7.55%. Methyl 6-oxoheptanoate, with a formula of C₈H₁₄O₃ and a weight of 158, contributes at 0.61%. dl-2,6-Diaminoheptanedioic acid, C₇H₁₄N₂O₄, weighing 190, comprises 0.97% of the composition. D-Glucose, 6-O- α -D-galactopyranosyl-, with a formula of C₁₂H₂₂O₁₁ and a weight of 342, constitutes 1.97%. α -D-Glucopyranoside, O- α -D-glucopyranosyl- (1 \rightarrow 3)- β -D-fructofuranosyl, C₁₈H₃₂O₁₆, with a weight of 504, is the most predominant, representing 79.72%. Other compounds include 3-Cyclohexen-4-ol-1-one, 3-tridecanoyl- (C₁₉H₃₂O₃), β -D-Glucopyranose, 4-O- β -D-galactopyranosyl- (C₁₂H₂₂O₁₁), Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans- (C₁₉H₃₆O₃), 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3 β ,5Z,7E)- (C₂₇H₄₄O₃), and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (C₃₅H₆₈O₅), contributing at various concentrations ranging from 0.46% to 4.77%.

DISCUSSION

The moringa amla millet bars are nutrient dense, and contain a significant amount of iron (57.5 mg). The bars were made with a harmonious blend of iron-rich ingredients such as pearl millet flakes, finger millet flakes, ground nuts, sesame seeds, and jaggery, along with Moringa leaf flour and Amla flours. Dried moringa leaves in powdered form contain a higher iron content (24 mg) compared to fresh leaves²². In addition, the moringa leaves used for making moringa leaf flour were sun-dried and not pretreated, making the product development process simple for common household preparations. Also, using the flaked form of millets increases protein content without altering the other macro- and micronutrients²³. The amla powder was used to supplement vitamin C (23.24 mg/100 g) because dried moringa leaves contain less vitamin C (0.1 mg/100 g) than fresh leaves (0.8 mg/100 g) to facilitate iron absorption²⁴. These findings denote that the Moringa amla millet bar is ideal for iron supplementation because it is produced at a low cost with minimal processing efforts. However, if moringa leaf flour is alkali-treated, it contains more bioactive phytochemicals and is suitable for ready-to-eat snack preparations with fewer anti-nutrient properties²⁵. Considering the acceptability of the product developed in this study, moringa flour incorporation (20%) is comparably higher than other products developed,

such as cakes, porridge, bread, and cookies, where only less than 15% was found to be acceptable²⁶. However, a study conducted with African species of moringa leaf flour in the preparation of chocolates stated that 20% moringa leaf flour incorporation was acceptable, which is similar to the findings of the present study (Abou-Zaid, 2014). The presence of millet flakes in the bar might have absorbed the grassy flavour of moringa leaf flour and resulted in a balanced taste²⁷. In addition, inferring from the overall and average product acceptability index for all three variants (80%) indicates that an incorporation of up to 60% moringa leaf flour may be acceptable. Therefore, a product with a higher concentration of moringa leaf flour and a perfect blend of millets and ground nuts, which are low-cost iron-rich ingredients, may be a better choice for iron supplementation because of its appealing flavour.

CONCLUSION

The results of the study conclude that the acceptability of the Iron Rich (Plant-Based Moringa Amla) Protein Energy Snack Bar depended on the ratio of Moringa Leaf Powder to Groundnut Flour. The lower the percentage of Moringa leaf powder, the higher the acceptability. The nutrient evaluation identifies that the Iron Rich (Plant-Based Moringa Amla) Protein Energy Snack Bars can be a better replacement as a healthy snack for adolescents, combating their micro- and macronutrient deficiencies and facilitating their health promotion and illness prevention.

ETHICAL APPROVAL

This study is part of the Randomised control trial on "Effectiveness of Adolescent Focused Intervention bundle on haematological parameters, emotional behavioural difficulties and resilience among adolescents living in child care institutions" and is approved by Institutional Ethical Committee SRM Medica College Hospital Research Centre. The trial is registered in clinical trial registry. The nutrient bar developed is to check its efficacy in improving the haematological parameters among adolescents as part of the trial.

ACKNOWLEDGEMENT

This paper is an output of the product development of a low-cost, nutrient-rich energy snack prepared by a research scholar full-time at SRM College of Nursing, SRMIST.

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