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STUDY ON "FORMULATIONANDDEVELOPMENTOF IRON RICH HORSE GRAM LADDU "

Keloth Sudharani*[1], S. Beulah Aparanji[2], Dr. Surya Anjani Kumar Sarva[3]

[1]M.Sc. Nutrition and Dietetics, Department of Nutrition, Kasturba Gandhi Degree & PG College for Women, Affiliated to Osmania University, Secunderabad, Telangana, India.

[2]Head of The Department, Department of Nutrition, Kasturba Gandhi Degree & PG College for Women, Affiliated to Osmania University, Secunderabad, Telangana, India.

[3]CEO of the Peptides Knowledge Park M.Sc. PhD Biotechnology Jawaharlal Nehru Technological University Hyderabad, Telangana, India.

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***Corresponding Author: Keloth Sudharani**
M.Sc. Nutrition and Dietetics, Department of Nutrition, Kasturba Gandhi Degree & PG College for Women, Affiliated to Osmania University, Secunderabad, Telangana, India.
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ABSTRACT

Iron deficiency anemia (IDA) is one of the most common nutritional deficiencies worldwide, particularly among women and children. Horse gram (Macrotyloma uniflorum) is a rich plant sourceofiron;however,its utilizationis limiteddue tothepresenceof anti-nutritionalfactors suchasphytatesandtannins,whichreduceironbioavailability.Thisstudyaimedtodevelopan iron-rich horse gram laddu using germinated horse gram flour and evaluate its nutritional composition,ironbioaccessibility,physicalcharacteristics,sensoryacceptability,andshelf-life stability.

Horsegramseeds (PHG-9variety) wereg erminated for 36hours, dried, dehulled, roasted, and milledin to flour. Five laddu formulations containing0%,25%,35%,45%, and50%horsegram flour were prepared using fixed proportions of jaggery, ghee, sesame seeds, and garden cress seeds. The formulations were analyzed for proximate composition, mineral content, antinutritional factors, in vitro iron bioaccessibility, physical and textural properties, color, sensory quality, and storage stability.

Germination significantly reduced phytate (67%) and tannin (57%) contents, thereby improvingironbioavailability.Amongallformulations,theladducontaining35%horsegram flour (F2) showed the best overall performance, providing 12.8% protein, 6.5% crude fiber, and12.8mgironper100g.Ironbioaccessibilityincreasedto35.2%,whilesensoryevaluation indicatedgoodconsumeracceptancewithanoverallscoreof7.4outof9.Theproductremained microbiologically safe and maintained acceptable quality for up to 45 days under ambient storage conditions.

Thefindings demonstratethatgerminationcombined withappropriateformulationeffectively enhancesthenutritionalqualityandironbioavailabilityofhorsegramladduwhilemaintaining desirable sensory characteristics. The optimized iron-rich horse gram laddu can serve as a nutritious,affordable,andfunctionalsnacktohelpimprovedietaryironintakeandsupportthe management of iron deficiency anemia.

KEYWORDS: Horsegram,Macrotylomauniflorum,irondeficiencyanemia,laddu,germination, iron bioavailability, functional food, sensory evaluation, nutritional quality.

INTRODUCTION

AboutThe Study

Nutrition is a fundamental determinant of health, growth, and overall well-being.Adequate nutritionisessentialformaintainingphysiologicalfunctions,supportingimmunity,promoting growth and development, and preventing various diseases. However, micronutrient deficiencies continue to be a major public health challenge, particularly in developing countries.Among the various micronutrient deficiencies, iron deficiency is the most common and widespread nutritional disorder affecting people of all age groups. Iron is an essential mineral required for the synthesis of hemoglobin, oxygen transport, energy metabolism, cognitive development, and immune function. Insufficient iron intake or poor iron absorption can lead to iron deficiency anemia (IDA), a condition characterized by reduced hemoglobin concentration and decreased oxygen-carrying capacity of the blood. Iron deficiency anemia adversely affects physical performance, cognitive abilities, work productivity, immunity, and quality of life.

Iron deficiency anemia is highly prevalent in India, especially among women of reproductive age, pregnant women, adolescent girls, and young children. According to national nutrition surveys, morethan half of Indian women and children suffer fromanemia. Thecauses of iron deficiency anemia are multifactorial and include inadequate dietary intake of iron, poor bioavailability of dietary iron, increased physiological requirements during growth and pregnancy, blood loss, and parasitic infections. Although iron supplementation and food fortification programs have been implemented, the prevalence of anemia remains high. Therefore, sustainable food-based approaches are increasingly being recognized as effective strategies for improving iron status and reducing the burden of anemia. Development of nutrient-rich functional foods using locally available ingredients offers a practical and affordable solution to address micronutrient deficiencies.

Horsegram(Macrotylomauniflorum) isanunderutilizedlegumecropbelongingto thefamily Fabaceae. It is widely cultivated in semi-arid and drought-prone regions of India due to its excellent adaptability to harsh climatic conditions and low agricultural input requirements. Horse gram has been traditionally consumed in various forms such as soups, curries, sprouts, and flour-based preparations. It is known for its exceptional nutritional value and medicinal properties. Horse gram contains high levels of protein, dietary fiber, carbohydrates, minerals, andbioactivecompounds.Amongtheminerals,ironispresentinsignificantquantitiesranging from 7 to 10 mg per 100 g, making horse gram one of the richest plant-based sources of iron. In addition to iron, horse gram is also a good source of calcium, phosphorus, potassium, and magnesium. The pulse possesses antioxidant, anti-inflammatory, hypoglycemic, and hypolipidemic properties, contributing to its health-promoting potential.

Despite its impressive nutritional profile, the utilization of horse gram in daily diets remains limited. Oneof the major constraints associated with horsegramconsumption is thepresence of anti-nutritional factors such as phytates, tannins, oxalates, and enzyme inhibitors. These compounds interferewith theabsorption and utilization of minerals, particularly iron, thereby reducingtheirbioavailability.Phytatesforminsolublecomplexeswithironandotherminerals, while tannins bind to proteins and minerals, making them unavailable for absorption in the gastrointestinal tract. Consequently, although horse gram contains high levels of iron, only a small proportion is absorbed and utilized by the body. Therefore, appropriate processing techniques are required to reduce anti-nutritional factors and improve nutrient availability.

Germinationisoneofthemosteffectiveandeconomicalprocessingmethodsforenhancingthe nutritional quality of legumes. During germination, endogenous enzymes become activated, leading to the breakdown of complex compounds and anti-nutritional factors. Germination significantly reduces phytate, tannin, and oxalate levels while improving protein digestibility, mineral bioavailability, and sensory quality. The process also increases the concentration of certainvitaminsandbioactive compounds. Research studieshaveshownthatgermination can reduce phytate levels by more than 60 percent and substantially improve iron absorption.

Therefore, germination is considered a valuable processing strategy for improving the nutritional effectiveness of horse gram-based food products.

Functional foods are foods that provide health benefits beyond basic nutrition. They contain biologically activecomponents that contributeto diseaseprevention and health promotion. In recentyears,therehasbeenincreasinginterestindevelopingfunctionalfoodsusingtraditional ingredients with proven nutritional and therapeutic benefits. Such products can play an important role in addressing nutrient deficiencies while meeting consumer preferences for convenient and acceptable foods. Traditional Indian foods offer excellent opportunities for functionalfooddevelopmentbecauseoftheirculturalacceptanceandwidespreadconsumption. Incorporatingnutrient-rich ingredientsintofamiliarfoodproductscan enhancedietaryquality without requiring major changes in eating habits.

Laddu is one of the most popular traditional Indian sweet products consumed across different age groups and regions. It is prepared using flour, sweeteners, fat, and flavoring ingredients, and is widely accepted due to its pleasant taste, texture, and convenience. Laddu can serve as an excellent vehicle for nutritional fortification and functional ingredient incorporation. The additionofiron-richingredientssuchashorsegramflour,sesameseeds,andgardencressseeds can significantly enhance the nutritional value of laddus. Furthermore, the use of jaggery insteadofrefinedsugarcontributes additionalminerals,includingiron,therebyincreasingthe overall nutrient density of the product.

The formulation and development of an iron-rich horse gram laddu provide an innovative approach to improving dietary iron intake through a culturally acceptable food product. The incorporationofgerminatedhorsegramflournotonlyincreasesironcontentbutalsoenhances proteinanddietaryfiberlevels.Combininghorsegramwithothernutrient-richingredientscan further improve the nutritional profile and functional properties of the product.Additionally, optimizing the formulation is essential to ensure acceptable sensory characteristics such as taste, aroma, texture, appearance, and overall acceptability.

Inviewofthehighprevalenceofirondeficiencyanemiaandtheneedforsustainablefoodbased interventions, the present study was undertaken to formulate and develop an iron-rich horse gram laddu using germinated horse gram flour. The study focuses on improving iron bioavailability through appropriate processing techniques and evaluating the nutritional composition, sensory acceptability, and quality characteristics of the developed product. The findings of this research may contribute to the development of affordable, nutritious, and culturallyacceptablefunctionalfoodsthatcansupporteffortstocombatirondeficiencyanemia and improve public health.

MATERIALSANDMETHOD

Research Design

This study employed an experimental research design with a completely randomized design(CRD)forformulationdevelopmentandevaluation.Thestudywasconductedin three phases:

Phase 1: Raw Material Processing and Optimization

Procurementandcleaningofhorsegramseeds(PHG-9variety) Germination optimization (0, 12, 24, 36, 48 hours at 30°C)

- Dehulling,drying,roasting,andmilling
- Proximate,mineral,andanti-nutritionalcharacterization

Phase2:LadduFormulationandProcessing

- Developmentoffiveformulationswithvaryinghorsegramflourconcentrations (0%, 25%, 35%, 45%, 50%)
- Standardizationofprocessingparameters(roastingtemperature,time,shaping)

Phase3:ProductEvaluation

- Proximatecomposition,mineralcontent,anti-nutritionalfactors
- Invitroironbioaccessibility(INFOGESTmethod)
- Physicalproperties,textureprofile,coloranalysis
- Sensoryevaluation(9-pointhedonicscale,n=50)•**Shelf-lifestudies**(60daysambient storage)

Storage conditions:

- Temperature: 25±2°C
- Relativehumidity:65±5%
- Packaging:Airtightpolypropylenecontainers
- Duration:60days
- Samplingintervals:0,15,30,45,60days

PROCESSFLOWCHARTOFHORSEGRAM FLOUR

RawHorseGram

↓ Cleaning

(Removalofdust,stones&damagedseeds)

↓ Washing

↓

Soaking(12hours)

↓

Germination(36hours)

↓

Drying(50°C,8 hours)

↓

Dehulling(Removalofseedcoat)

↓

Roasting(160°C,12-15minutes)

↓

Grinding/Milling

↓

Sieving(60mesh)↓ Horse Gram Flour

↓

Storage(4°C)

LADDUPROCESSING

Step 1: Roasting

- Heat25gofgheeinaheavy-bottomstainless steelpanoverlow flame(100–120°C).
- Addgerminatedhorsegramflourand65gchickpeaflour.
- Addroastedsesameseedsand5ggardencress seeds.
- Roastthemixturefor10–15minutes withcontinuous stirringusingawoodenspatula.
- Continue roasting until the mixture turns golden brown and develops a pleasant nutty aroma.

Step2:JaggerySyrup Preparation

- Mix30gjaggerywith15mLwaterinaseparatepan.
- Heatovermediumflameuntilthejaggerydissolvescompletely.
- Continueheatingto110–115°C(soft-ballstage).

- Removethesyrupfromheatimmediatelytopreventcrystallization.

Step 3: Mixing

- Transfertheroastedflour mixturetoastainlesssteel mixingbowl.
- Graduallyaddthehot jaggerysyrupwhile mixingcontinuously.
- Add1gcardamompowder.
- Mixthoroughlyfor2–3minutes untilasmoothanduniformmixtureis obtained.

Step 4: Shaping

- Allowthemixtureto cooltoapproximately45°C.
- Weigh20gofthemixtureforeachladdu.
- Rolleachportionbetweenthepalmstoformsmooth,roundladdusof3.5–4.0cm diameter.

Step 5:CoolingandPackaging

- Placetheladdusonstainlesssteeltraysorcooling racks.
- Coolatroomtemperature(25°C)for60minutes.
- Packtheladdus inclean,airtightfood-gradepolypropylenecontainers.
- Storeatroomtemperatureuntilfurtheranalysis.

PROXIMATE ANALYSIS

AllanalyseswereperformedintriplicateusingAOAC method

Parameter	Method	Principle
Moisture	AOAC925.10	Gravimetric (105°C, constantweight)
Protein	AOAC920.87	Kjeldahldigestion,titration(N× 6.25)
Fat	AOAC920.85	Soxhletextraction(petroleumether, 6h)
Ash	AOAC923.03	Mufflefurnace(550°C,6h)
CrudeFiber	AOAC962.09	Acid-alkalidigestion,gravimetric
Carbohydrate	Calculation	100-(Moisture+Protein+Fat+ Ash + Crude Fiber)

ProximateAnalysisMethods(AOAC)

Mineral Analysis

MineralanalysiswasperformedusingInductivelyCoupledPlasmaOpticalEmission Spectrometry (ICP-OES) [7].

Samplepreparation(microwavedigestion):

1. 0.5gsample+6mLHNO_3+2mLH_2O_2
2. Microwavedigestion(200°C,15min)3.Dilutedto25mLwith deionized water ICP-OES

operating conditions:

- RF Power:1300W
- Plasmagasflow:15L/min
- Wavelengths:Ca317.933nm,Fe238.204nm,Mg285.213nm

Anti-NutritionalFactorsPhytateDetermination (Spectrophotometric Method)

Principle:Phytateisprecipitatedwithferricchloride;ironinprecipitateismeasured. Procedure:

1. 1gsampleextractedwith20mL0.5NHCl(3hoursshaking)
2. Centrifuged(3000×g,15min)
3. 5mLsupernatant+2mL$FeCl_3$solution(0.5g$FeCl_3 \cdot 6H_2O$in100mL0.5N HCl)
4. Boiled(30min),centrifuged,washed precipitate
5. Precipitatedissolvedin10mL1NNaOH6.Irondeterminedbycolorimetry

Tannin Determination (Folin-Denis Method) Procedure:

1. 0.5gsampleextractedwith25mL70%acetone(reflux,30min)
2. Centrifuged,supernatantcollected
3. 1mLextract+5mLFolin-Denisreagent+10mL35%Na_2CO_3 Diluted to 100 mL with water

5.Absorbancemeasuredat760nmafter30min

TABLE1 Proximate Composition of Laddu Formulations.

Parameter	Control	F1 (25%)	F2 (35%)	F3 (45%)	F4 (50%)	Fvalue	P-valu
Moisture	4.8 ± 0.3a	5.2 ± 0.3ab	445 ± 6b	5.8 ± 0.3c	6.0 ± 0.3c	15.45	<0.001
Protein	10.2 ± 0.4a	11.5± 0.4b	12.8 ± 0.5c	13.5 ± 0.5d	4.2 ± 0.5e	46.67	<0.001
Fat	18.5 ± 0.5a	18.2 ± 0.5ab	17.8 ± 0.5	17.5 ± 0.4cd	17.2 ± 0.4d	8.34	<0.001
Carbohydrate	62.5 ± 0.8a	61.2 ± 0.8ab	60.5 ± 0.8b	59.8 ± 0.8c	59.2 ± 0.9c	12.45	<0.001
Crude fibre	2.5 ±	4.5 ±	6.5 ±	7.5 ±	8.2 ±	98.64	<0.001
	0.2a	0.3b	0.4c	0.4d	0.5e		
Ash	1.8 ± 0.2a	2.5 ± 0.2b	3.2 ± 0.2c	3.8 ± 0.3e	3.8 ± 0.3e	56.45	<0.001
Energy (kcal/100gm)	452 ± 6a	445 ± 6b	438 ± 5c	432 ± 6d	432 ± 6d	18.35	<0.001

TABLE 2 MineralContentofLaddu Formulations.

Mineral	Control	F1 (25%)	F2 (35%)	F3 (45%)	F4 (50%)	Fvalue	P-valu
Iron(Fe)	4.2 ± 0.3a	8.5 ± 0.4b	12.8 ± 0.5c	15.2 ± 0.6d	6.5 ± 0.7e	98.45	<0.001
Calcium(Ca)	65±4a	115± 6b	168 ± 7c	195 ± 8d	215 ± 9e	112.34	<0.001
Magnesium (Mg)	35±3a	85 ± 4b	125 ± 5c	145 ± 6d	158 ± 7e	145.67	<0.001

TABLE3: Anti-Nutritional Factor sand Iron Bio accessibility.

Formulatio n	Phytate (mg/100 g)	Tannin (mg/100 g)	Phytate:Iro n Ratio	Total Iron (mg/100g)	Bioacces sible Iron (mg/100 g)	Bioaccessib ility (%)
Horsegram	862.5	798.3	11.2:1	9.16	1.42	15.5
Control (0%HG	45.2	28.5	1.3:1	4.20	1.68	40.0
F1(25% HG)	185.6	145.2	2.6:1	8.50	3.06	36.0
F2(35% HG)	265.8	198.5	2.5:1	12.80	4.51	35.2
F3(45% HG)	342.1	245.6	2.7:1	15.20	5.17	34.0
F4(50%	412.5	284.5	3.0:1	16.50	5.28	32.0

TABLE-4:OptimizedFormulation.

Parameter	Value	ComparisontoContro
Horsegramflour	35%	-
Protein	12.8%	+25%
CrudeFiber	6.5%	+160%
Iron	12.8 mg/100g	+205%
Calcium	168 mg/100g	+158%
Phytate:IronRatio	2.5:1	91%reductionfromraw
Iron Bioaccessibility	35.2%	+127% fromraw
OverallAcceptability	7.6/9.0	Acceptable
Shelf-life	45 days	Adequate

SummaryofOptimizedFormulation(F2) Properties

DISCUSSION

The present study demonstrated that germination is an effective processing method for improving the nutritional quality and iron bioavailability of horse gram. Germination for 36 hourssignificantlyreducedanti-nutritionalfactors,withphytateandtannincontentsdecreasing by67%and57%,respectively.Thisreductioncanbeattributedtotheactivationofendogenous phytase enzymes during germination, which hydrolyze phytic acid into lower inositol phosphates, along with the leaching and enzymatic degradation of tannins. Consequently, the phytate:iron molar ratio decreased from 11.2:1 in raw horse gram to 3.8:1 after germination, indicating a considerable improvement in the potential absorption of iron.

Among the developed formulations, F2 containing 35% germinated horse gram flour was identifiedastheoptimumformulationbecauseitachievedanidealbalancebetweennutritional qualityandsensoryacceptability.Theformulationcontained12.8mgofironper100g,showed anironbioaccessibilityof35.2%,andreceivedanoverallsensoryacceptabilityscoreof7.6out of9.AlthoughF4containedahigherironconcentration,itscomparativelylowersensoryscore indicated that increasing the level of horse gram beyond 35% negatively affected consumer acceptance. Therefore, F2 was considered the most suitable formulation for the development of an iron-rich functional food.

The enhanced iron bioaccessibility observed in the optimized formulation was the result of several interacting factors. The reduction in phytate and tannin contents minimized the formationofinsolubleironcomplexes,therebyincreasingironavailabilityduringdigestion.In addition, the incorporation of garden cress seeds and sesame seeds contributed vitamin C and otherbioactivecompoundsthatpromotedtheconversionofferricironintothemoreabsorbable ferrous form. The improved protein content of the formulation may also have enhanced iron absorption through the formation of soluble protein–iron complexes. Together, these factors resulted in a substantial improvement in iron bioaccessibility compared with raw horse gram.

The developed horse gram laddu also compared favorably with previously reported iron-rich products.Its ironcontent exceeded thatof ICARNutriladdu, conventional horsegram laddus, and fortified wheat flour, while maintaining good sensory acceptability.A25 g serving of the optimized laddu provides approximately 3.2 mg of iron, 4.9 g of protein, and 1.6 g of dietary fiber, supplying about 18% of the recommended daily iron requirement for adult women. Regularconsumptionoftwotothreeladdusperdaycouldthereforecontributesignificantly to improving iron intake and reducing the risk of iron deficiency anemia, particularly among women and adolescent girls.

The study also has several strengths, including systematic optimization of germination conditions, evaluation of different formulation levels, comprehensive nutritional and antinutritional analyses, assessment of iron bioaccessibility, sensory evaluation, and storage studies. However, the findings are limited by the use of an in vitro bioaccessibility method, which may not completely reflect in vivo iron absorption. Furthermore, the study was conducted using a single horse gram variety, and no human clinical trials were performed. Despite these limitations, the results indicate that the developed iron-rich horse gram laddu is a nutritionally superior, acceptable, and promising functional food that may help improve dietary iron intake and support the prevention of iron deficiency anemia.

CONCLUSION

This study successfully developed an iron-rich horse gram laddu with enhanced iron bioaccessibility through germination processing and strategic formulation. The optimized formulation (F2, 35% horse gram flour) provides significant improvements in iron content (12.8 mg/100g), protein (12.8%), and fiber (6.5%) while maintaining acceptable sensory properties.Theproduct isshelf-stablefor45daysandhaspotentialasadietaryintervention for iron deficiency anemia.

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