"DEVELOPMENT OF AN IRON-RICH LADOO-FOR THE PREVENTION OF IRON DEFECIENCY ANEMIA"

PROJECT WORK

SUBMITTED TO DEPARTMENT OF PG STUDIES IN FOOD SCIENCE AND NUTRITION,

BESANT WOMEN'S COLLEGE, MANGALORE

IN THE PARTIAL FULFILMENT OF REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

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CERTIFICATE

This is to certify that the project work entitled "**DEVELOPMENT OF IRON RICH LADOO- FOR THE PREVENTION OF IRON DEFECIENCY ANEMIA"** is an authentic record of independent research work done by **Ms.AMEERA(Register number: 193041961)** under my supervision during the period of **July 2021 to October 2021**, submitted to

Mangalore university for the partial fulfilment for the award of the degree of **Master of Science In Food Science And Nutrition** and the present work has not been previously formed the basis for the award of degree, diploma, fellowship, associateship or other titles.

Place: PATHOOR

DATE: 30 OCTOBER 2021

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CERTIFICATE

This is to certify that the project work entitled **"DEVELOPMENT OF AN IRON RICH LADOO- FOR THE PREVENTION OF IRON DEFICIENCY ANEMIA** submitted to the **Department of PG** Studies **in Food Science and Nutrition, by Mrs. AMEERA (Reg.no:193041961)** towards the partial fulfilment of the degree of **Master Of Science In Food Science And Nutrition**, is a faithful record of original work carried out by her in the academic year **2020-2021.**

Place: PATHOOR

DATE: 30 OCTOBER 2021

HEAD OF THE DEPARTMENT

EXAMINERS:

1.

2.

DECLARATION

I **AMEERA** hereby declare that project work entitled **"DEVELOPMENT OF AN IRON RICH LADOO- FOR THE PREVENTION OF IRON DEFICIENCY ANEMIA"** is a bona fide record of research work done by me under the supervision of **Mrs. ROOPA RAO K Besant**, **Assistant**

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The information depicted in the current report is the result of my own work, except where the reference is made. The information provided in the report is authentic as per my knowledge.

The results embodied in the project work have not been submitted to any other university or institution for the award of any degree, diploma, associateship, fellowship or similar titles.

Place: PATHOOR

DATE: 30 OCTOBER 2021

AMEERA

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ABSTRACT

Iron deficiency anemia is one of the most common nutrient deficiencies in the world, which occurs due to insufficient iron in our bodies. So, it is important to incorporate iron-rich foods into our diet for the prevention of iron deficiency anemia. Pre-school, school children along with pregnant women are at high risk for developing iron-deficiency anemia. So, an iron-rich ladoo was formulated using iron-rich ingredients like Garden cress seeds, Niger seeds, Rice flakes and Jaggery. The developed ladoo was rich in iron as it contained 18.43 mg of iron per 100 grams of sample. The shelf life of the product was 14 days. The moisture content of the ladoo was within the acceptable level and it was also rich in fibre which provides bulk to the diet. Since it was formulated as ladoo, which is a sweet snack, it can be easily incorporated into the daily diet of preschool, school children and teenagers for the prevention of Iron deficiency anemia.

CHAPTER 1

INTRODUCTION

Iron deficiency anemia is one of the most common nutrient deficiency problems in India. It is caused due to poor iron intake in the diet, gastrointestinal malabsorption or due to occult blood loss. It is characterized by a reduced level of iron in the blood which results in the manifestation of symptoms of iron deficiency anemia. Iron deficiency anemia is usually chronic and asymptotic, so they get undiagnosed. Non-specific symptoms include fatigue, difficulty in concentration, weakness, less work productivity is attributed to decreased delivery of oxygen to body tissues along with the reduced activity of iron consisting enzymes. In children, a decrease in cognitive function and delayed motor development are observed. In pregnancy, iron deficiency anemia can lead to low neonatal weight, premature delivery and an increase in newborn and maternal mortality rates.

The prevalence of iron-deficiency anemia is high in developing countries. Infants, young children, women of childbearing age and adults with internal bleeding are at high risk of developing iron-deficiency anemia. Since one of the main causes of iron deficiency is poor intake of iron in the diet, it can be corrected by food-based approaches such as the development of iron-rich food products. Our body requires iron and other nutrients to form hemoglobin and red blood cells. So it is important to get a regular supply of iron through our diet.

India is a country that is rich in natural resources and the majority of the population are vegetarians(which has decreased bioavailability), so consumption of iron-rich foods immensely help in the prevention of iron deficiency anemia. An iron-rich ladoo was developed using iron-rich ingredients like Garden cress seeds, Niger seeds, Rice flakes and Jaggery.

Garden cress seeds:

Garden cress seeds (*Lepidium sativum.L.,*) commonly known as Hadim seeds, Land cress seeds and Haliv in India. It is considered to be an important medicinal crop. They

are used in treating many health conditions like asthm**a,** diarrhea, cough with expectoration, dysentery, skin disease, scurvy etc.

Garden cress seeds are an important source of iron, calcium, vitamin A, C, E and folic acid. It is the richest source of iron as 100 grams of seeds contains 100 mg of iron. Studies show that consumption of this seed for 1-2 months helped immensely to increase hemoglobin levels. So garden cress seeds were used for the development of iron-rich ladoo.

Niger seeds:

Niger seeds(*Guizontia Abyssinia)* is an oilseed crop cultivated in India. They are commonly known as Kala til, ram til and sorguja. They are a good source of energy, protein, iron and linoleic acid. Niger seeds due to their high level of vitamin k1 help in the blood clotting mechanism, the high content of linoleic acid helps in lowering LDL cholesterol which in turn helps in the prevention of cardiovascular disease and arrhythmias. They are also used to treat syphilis(belay eh, 1991) and rheumatoid arthritis.

Niger seeds due to their high iron content also helps in the prevention of anemia. This seed provides 56.7 mg of iron/100 grams(Gopalan *et Al*, 2007), which is high when compared with other oilseeds. So, Niger seeds were also incorporated as the second main ingredient in the development of iron-rich ladoo.

Rice flakes:

Rice flakes is a commonly used breakfast cereal prepared from paddy and has been the base product of India. They are commonly known as Poha, avalakki, aval, chivda and beaten rice. They are rich in iron(20 mg) and calories(346 kcals)[17]. Rice flakes are also rich in vitamin C which boosts iron absorption. So iron-rich ladoo was developed using rice flakes along with other ingredients.

Jaggery:

Jaggery is a traditional Indian sweetener commonly known as ‘Gur' in India and is produced from sugarcane. They are a natural mixture of molasses and sugar. They are easily available and a good source of iron, i.e. 100 grams contains Jaggery contains 3 mg of iron. A considerable amount of ferrous salt is also gathered by jaggery during its preparation in an iron vessel. The Health benefits of Jaggery includes helping in the proper functioning of the nervous system, blood pressure and heart function regulation, improvement of digestion and strengthening of lungs and bones. In *Sushruta Samhita*, it is written that Jaggery purifies the blood. So all these health benefits and high iron content are taken into consideration and jaggery was selected as a sweetener for the recipe.

Processing methods:

In the formulation of iron-rich ladoo, different processing methods like roasting, soaking and cooking in iron vessels were used. These methods help in reducing the level of different anti-nutrients. Rice flakes and Niger seeds were subjected to dry roasting. Garden cress seeds were soaked before incorporating them into the recipe. Soaking helps in reducing the level of phytic acid and raffinose (Akinyele *et al.*, 1989). Jaggery was melted in an iron Kadai to enhance their iron content. Cooking food in an iron vessel is known to improve the iron status of food[23].

CHAPTER 2

REVIEW OF LITERATURE

2.1. Prevalence of iron deficiency anemia

Ramesh Chellan *et al.*, (2010) studied the prevalence of Iron Deficiency Anemia in India using survey data obtained from the blood hemoglobin level of a study group consisting of 311, 793 adolescents, 430,626 children and 41, 112 pregnant women. The outcome of this study showed that more than 95% of pregnant women, adolescent girls and children were found to be anemic, among which the prevalence rate of highest was observed in adolescents(97.8 %), especially adolescent girls where 27.1% of them had severe anemia. Among pregnant women and children, nearly half of them had mild anemia, 42.6 % of pregnant women had moderate anemia and 2.9% of children had severe anemia. All these facts confirm the highest prevalence of anemia among the population of India.

Bhimrao N Jadhav (2016) reviewed on how Indian female population are affected by iron deficiency anemia especially during their parturiency phase which has a high demand for iron and folic acid. The main reasons for iron deficiency anemia which can affect both mother and infant's health are insufficient iron intake, increased demand for iron and blood loss. Dietary approaches like the inclusion of a sufficient amount of high bioavailable iron along with dietary diversification, fortification of food with iron and supplementation of iron and vitamins should be considered not only for pregnancy but also for all other age groups to manage iron deficiency anemia.

Lisa M Sinclair *et al.*, (2005) performed a study to determine the prevalence of iron deficiency with or without anemia among 121 aerobically trained both male(49) and female(72) adults of age group 18-41 years. Iron deficiency anaemia is known to reduce working capacity(Vo2 max, maximal oxygen use) due to inadequate oxygen transport by hemoglobin to peripheral tissue. The subjects were screened for their iron status using the concentration of hemoglobin, ferritin and transferrin receptors which showed that 8 subjects (1 male, 7 females) had iron deficiency with anaemia(Hb<120 g/L for female, Hb<136g/L for male and ferritin <16 microgram/L) while 29% of female and 4% of male subjects had iron deficiency without anemia(ferritin ≤16 microgram/L). Detection of iron deficiency without anemia using transferrin receptor-ferritin

index(serum transferrin receptor: log serum ferritin ≥4.5) showed that 6% of male and 36% of female had iron deficiency without anemia. Result of this study reveals that even males are not immune to iron deficiency and comparatively, prevalence is greater in trained females than males.

2.2. Effect of iron deficiency anemia

Sarika More *et al., (*2013*)* assessed how a deficiency of iron can affect the cognitive function of adolescent school-going girls living in the rural area of central India. Girl students of age 12-15 years were selected as subjects and they were screened for iron deficiency and anemia using complete blood count. This test showed a prevalence of 63% of anemia in school going, adolescent girls. For evaluation of cognitive function score Scholastic assessment, multicomponent test, PGI test and Intelligent quotient tests were conducted on subjects which revealed that iron-deficient anemic and the non-anemic subjects had less cognitive function score when compared with non-anemic non-iron deficient subjects.

Lindsay H Allen (2000) discusses how iron deficiency can affect pregnancy. It can cause premature delivery, low birth weight and poor neonatal health. There is no sufficient data that can help to determine the extent of maternal anemia that can lead to maternal mortality, which just shows that there are many gaps in our understandings of adverse effects of maternal iron deficiency and anemia. Many pieces of evidence confirm that maternal anemia can decrease fetal iron stores and they also stress on implementation of routine iron supplementation, especially during pregnancy.

2.3. Iron bioavailability

K.K.sharma (2003) discussed how the National tenth plan aims to control iron-deficiency anemia by enhancing iron bioavailability in the Indian meal via food-based approaches such as fortification of food, diverseness of diet, folic acid and iron supplementation along with schemes to prevent parasitic invasions in agriculture. Various factors like non-haem iron, phytates and polyphenols, calcium are known to reduce the bioavailability of iron. Food approaches such as the use of ascorbic acid(which reduces the ferric iron to the more absorbed form by 75-98%), production and distribution of more foods that are iron-rich; enhancers of iron bioavailability and their consumption with a suitable combination along with the use of methods like

fermentation, germination and malting of pulses and grains that helps in reducing phytate level and enhancing ascorbic acid can be used for controlling iron deficiency anemia.

Richard Hurrel *et al.*, (2010) investigated iron bioavailability using intake data and isotope studies conducted on the subjects without iron stores, which showed a 5-12% of iron bioavailability for vegetarian diets and 14-18% for mixed diets. Factors like polyphenols, phytates, ascorbic acid, calcium and muscle tissues were shown to improve iron absorption in single-meal isotope studies, but in the multi meal study with a mixed diet involving many enhancers and inhibitors, the influence of a single component was at a limited level. The significance of iron fortification and food additives like erythrobic acid on the bioavailability of iron from the mixed diet and influence of carotenoids, vitamin A and indigestible carbohydrates on the absorption of iron and the type of "meat factor" is not yet clearly understood. Other host factors like obesity and iron status of the individual(which has creator effect than the composition of diet) have a significant role in iron bioavailability. So it is important to create a range of iron bioavailability factors not only based on the composition of diet but also based on the characteristics of subjects like the prevalence of obesity and the status of iron.

2.4. Iron-rich product development

Monika Jain (2013) compared the impacts of both the iron-rich recipe and medicinal iron supplementation on three groups of anemic subjects where the 1st group was instructed to have iron and folic acid supplements and 2nd group with the iron-rich recipe(using ingredients like Niger seeds, defatted soy flour biscuits, plus two lemons) and 3rd group without any supplement for 120 days. Iron-rich biscuits added with Niger seeds had great acceptability along with 10.8mg% iron. Hemoglobin was estimated before and after supplementation which revealed that iron-rich food supplementation was effective to a marginal extent when compared with medicinal iron supplementation whereas the impact of iron-rich food recipe on hemoglobin remained for 4 months but same was not observed in the case of iron supplementation indicating that iron-rich food supplementation as a good strategy to combat iron deficiency anemia in school going girls.

Mousmee Sood *et al.*, (2002) developed an iron-rich supplement for controlling iron deficiency anemia in 7-9 years school going children of low income family using regionally available ingredients like jaggery, garden cress seeds, processed rice flakes and amaranth seeds(45:40:10.5). The product *laddoo* which was given one per day to the selected 36 children having hemoglobin level below 11 g/dL for 60 days showed a significant increase in the hemoglobin level of 7-8 years age group than 8-9 years without any improvement in their height and weight. The developed product provided around 39 mg% of iron. These findings show that iron supplementation through food can be a good approach to reduce anemia in school-going children.

Pragya Singh *et al.*, (2007) developed iron-rich biscuit mixes using finger millet and other regionally available ingredients or by fortifying them using chemicals like Fe2So3 and they were evaluated for their proximate composition, Calcium, Phosphorus, Iron, in-vitro protein digestibility, invitro iron bioavailability, anti-nutrients, storage stability, sensory quality and acceptability. The developed final product had incorporation of finger millet up to 70% with nutrients(10.90-24.14% of crude protein, 0.30-3.70% of crude fat, 0.60-4.70% of total ash, 24.07 mg% of iron, 22.68-42.00% of invitro iron bioavailability, 22.00- 552.37 mg% of calcium and 119.80-372.30 mg of phosphorus) and they were inexpensive, storage-stable, acceptable at the field and laboratory level. So these iron-rich mixes can be very useful in nutrient supplementation programs.

Sneh Singh *et al., (*2019) developed an iron-rich muffin using sunflower seeds(a rich source of iron, essential fatty acids, vitamins and minerals), sesame seeds and watermelon seeds(rich in iron and protein) in 4 different variations(A, B, C, D) each consisted 10% of three different seeds(A-water melon seeds, B-water melon seeds, C-Sesame seeds) and variant D consisted of 3.3% of all the 3 seeds and then they were evaluated for their acceptability. The development of the product(iron-rich muffin) involved 5 phases: a)Raw materials collection and product preparation, b)Acceptability evaluation using 9 points hedonic scale, c)Proximate analysis, d)Testing of phytochemicals(saponins, flavonoid, tannins and total phenols), vitamin C and shelf life, e)Statistical analysis of 4 variants. Sensory, nutrient and phytochemical evaluation of products showed that among 4 different variations, variant D had a good score, high acceptability and was nutritious when compared with a standard muffin. Since this product contains all types of micronutrients and macronutrients, it can be very helpful for controlling iron deficiency anemia, especially in adolescents and children.

Uma Rani M *et al.*, (2016) formulated and standardized iron-rich laddoo incorporating an iron-rich ingredient, garden cress seeds(*Lepidium sativum*) which provides 100mg of iron/100g of seeds. The samples were prepared in 2 different variations. The nutrient composition standardized for fifty grams of laddoo and nutrient calculations of 2 samples showed that, of the two samples; sample 2 had a high iron content when compared with sample 1, as more amount of garden cress seeds were added in that sample. The organoleptic evaluation using 5 point hedonic scale showed that sample 1 had high acceptability than sample 2. So, this product developed as a convenient snack for adolescents can be very beneficial to them as it contains a high amount of iron and protein and it can also help in preventing iron-deficiency anemia.

Leva Alaunyte *et al.*, (2014) performed a 6-week dietary intervention study on 11 recreational female runners(age 32±7 years;239±153 mins exercise/week, of which16±150mins running activity/week; vo2 max 38±4 ml/kg/min) for the improvement of their iron status. Participants were instructed to substitute their usual bread with iron-rich Teff bread in their daily diet and dietary habits were analysed by multiple pass 24-hour recalls and venous blood assessment for serum transferrin, serum ferritin, serum transferrin receptor, total iron-binding capacity and transferrin receptor/ferritin log index was used for the measurement of iron status. Pre-intervention study of all subjects showed insufficient dietary intake of 10.7±2.7 mg/day, and over a third of all the subjects had depleted iron stores in the body(serum ferritin<12microgm/L) and macronutrient assessment showed sufficient energy, protein and fibre intake but saturated fat and total fat was above the required level. The post-intervention study(6 weeks) revealed a significant increase in total iron intake(18.5mg/day $p<0.05$) along with an improved supply of iron tissue. Improvement of haemoglobin indices is related to prolonged intervention time, compromised baseline iron status and increased iron intake. So the improvement in the dietary iron intake and total iron status of physically active females can be done by using an iron rich staple cereal product.

Radha Banka *et al.*, (2017) developed value added iron-rich products using underused dried leaves of Cauliflower(71.45 mg of iron/100gm), Carrots(73.6 mg of iron/100gm), beet green(83.6mg of iron/100gm), turnip(83.25mg/100gm) and also curry leaves(10mg/100gm). The 4 types of products *Chakli*, *Namakpara*, tarts and *Bhujia* were developed with 5%, 10% and 15% of incorporation of dried leaves for their iron

content, of which 5% level of incorporation had the highest acceptability 10% and 15% had the least acceptability with the notable differences(P>0.001) for all the 4 products. So this low cost and iron-rich products can be used as dietary iron supplements to prevent iron deficiency anemia in our community.

2.5.Rice flakes

Reenu Rana *et al.*, (2019) formulated products with improved iron status by incorporation of rice flakes powder. Rice flakes are rich in iron, carbohydrates, protein and calcium. Developed *laddoo* and *sev* recipes were standardized and sensory evaluation was conducted which revealed that the product was desirable in all terms. Nutritional evaluation of product showed the high iron content of supplement product *laddoo* 8.9±0.07 and *sev* 5.3±0.27. This kind of high iron content products can be very efficient in controlling iron deficiency anemia.

R.C Suma *et al.*, (2007) studied on the effect of dietary fibre and phytic phosphorous on in vitro iron and calcium bioavailability from rice flakes of four different thicknesses ranging from 0.08-1.20mm using equilibrium dialysis and standard methods. Final findings of this study disclosed that rice flakes had a mineral content ranging from 0.05-1.2 g, calcium content 107-210mg/100g and iron content 3.38-6.86 mg while phosphorus(111-430mg/100g), phytin phosphorus(23-164mg/100g) and dietary fibre (5.64-11.5g/100g) were shown to decrease with the degree of flaking. Bioavailability of iron and calcium in rice flakes (from thick to thin) were ranging from 7-26% and 8-25% respectively. Multiple regression analysis of the data revealed an important association of dietary fibre and phytin phosphorus in calcium and iron bindings.

2.6. Garden cress seeds(*Lipidium Sativum. L)*

Tarvinderjeet Kaur *et al., (*2015) investigated the enrichment of Indian traditional food with garden cress seeds which are a rich source of iron, protein and beta vitamin, to control iron deficiency anemia. Organoleptic evaluation using Hedonic nine-point scale was conducted to determine product acceptability and analysis of variance(ANOVA) was used for data interpretation which showed that among recipes incorporated by roasted garden cress seeds, *atta besan laddoo* had the best acceptability and *matrey* had the least acceptability, whereas among recipes incorporated by soaked

garden cress seeds, sweet and sour vegetable had high acceptability and salty *lassi* had the least acceptability. Increase in the iron content ranging from 79.3%(*shakarpara*) to 311.6%(*mathri* and *matrey*) in the roasted garden cress seed incorporation and 37%(sweet and salty *lassi* and cool drink) to 500%(*kheer*) in the soaked garden cress seeds were observed which confirms that enrichment of food with garden cress seeds can improve their iron status.

Chandra Shekar Singh *et al.,* (2017) examined the potentiality of garden cress seed(*Lipidium Sativum. L)*, a medicinal plant which belongs to the Cruciferae family, in the development of functional foods. Study of biologically active compounds and antioxidants capacity of garden cress seeds showed that it is a rich source of amino acids, fatty acids, minerals and phenolic compounds(which makes them act as antioxidants both in *in-vitro* and *in vivo)*. The ability of the garden cress seeds to act as a galactagogue, emmenagogue, gastrointestinal tract cleansing agent, haematic agent, a rich source of iron, alpha-linoleic acid and other nutrients makes it consider as a prime ingredient for the formulation and fortification of healthy functional foods.

2.7. Niger seeeds *(Guizotia Abyssinica*)

Rohini Jain *et al.,* (2016) developed iron-rich products(*atta laddoo*, *mathi* and salty biscuits) incorporating Niger seeds and evaluated their quality. The Organoleptic evaluation of products using an 8 point hedonic scale showed that products incorporated with 20% Niger seeds were acceptable and proximate composition analysis of the products revealed that it contained protein(13-15%), fibre(4.5-5.7%), fat(20-31%) and iron(11.8-16.1 mg/100g) which was higher than the control(0% supplementation). The products added with Niger seeds when measured for their protein digestibility showed a notable increase($p < 0.05$) in their digestibility. These nutritious products were developed as a dietary approach to control malnutrition and iron deficiency anemia.

Deepika Baranwal *et al., (*2013) studied the impacts of different processing techniques such as soaking, germination and roasting on the iron, bioavailable iron and anti-nutrients(phytic acid) of Niger seeds(*Guizotia Abyssinica*) which is an underutilised rich source of iron. The result of this study revealed the iron content of raw(control), soaked, germinated and roasted Niger seeds in the range of 38.56-42.43 mg/100g. Out of all the processing techniques, germination of Niger seeds were shown to increase the bioavailability of iron and decrease the phytic acid level(52.6%). So this information

can be very useful for increasing the bioavailability of iron during the formulation of iron-rich products.

2.8. Jaggery

Sindhu S *et al.*, (2013) studied the effectiveness of *Moringa Oleifera* leaves(in the form of non-heme iron of vegetable origin) and Jaggery in treating iron-deficiency anemia. Subjects selected were 60 women with iron deficiency anemia(diagnosed using Tallquist's hemoglobin scale), of which 30 of them were taken as intervention group supplemented with 100 gram of *M.Oleifera* and jaggery(dry weight) in the ratio of 80:20 for 30 days and the remaining 30 women were assigned as a control group. Post-intervention hemoglobin analysis showed a significant increase of hemoglobin level in the intervention group along with student's t test showing, $t = 4.109$ ($P<0.001$) which was used for determination of differences between intervention and control group. This study confirms that Moringa leaves and jaggery can be a good supplementation to the women with iron deficiency anemia.

Limna M (2018) investigated the influence of rice flakes and jaggery mixture consumption on the hemoglobin level of adolescent girls. The study was conducted on 30 adolescent girls of Pandalam Panchayat having hemoglobin level less than12 g/dL(selected using purposive sampling technique) using the king's goal attainment theory as a frame of reference. Selected subjects were supplemented with 150 g of rice flakes and jaggery mixture *laddoo* for 3 weeks and then tested for their hemoglobin level using Cyanmeth hemoglobin method. Data analysis by descriptive and inferential statistics showed an increase in the hemoglobin level from 11.33 (average mean pre-test with SD=0.45) to11.42 (average mean post-test with SD=0.44) and t value (8.31) was higher than table value(2.05) at *df* 29. All these findings indicate that rice flakes and jaggery mixture($P<0.05$) was effective in increasing hemoglobin level of adolescent girls and this study also discovered the relationship between pre-test hemoglobin level, the main source of information on anemia, subject's age and menstrual irregularity history in the previous year.

2.9. Cooking method(to improve iron content)

P.D Prinsen Geerlings *et al.*, (2003) reviewed about how cooking in iron pots can help in controlling iron deficiency anemia in developing countries. Data’s were analysed

from the Cochrane database of systematic reviews, the Cochrane controlled trial register, the database of abstracts of reviews effectiveness, Cochrane methodology register, Medline(1996-May 2002), Healthy technology assessment database & NHS economic evaluation database(Cochrane library issue 3,2002), the reference list of published trials and finally, the trial which compared the effect of cooking food in a non-cast iron pot and cast iron pot on participants for 4 months was selected in which one reviewer implemented the inclusion criteria to the relevant trials and 2 reviewers analysed the quality of trial and extracted data. So final findings from data showed some evidence pointing that consumption of food cooked in an iron pot can increase the hemoglobin concentration of iron-deficient individuals and this effect can vary according to the factors like consumer's age, presence of malaria; hookworm, size of the cooking pot, selected user group, familiarity with Iron cast pots and if the pot used is an extra or replacement pot etc. So, the introduction of cooking in iron pots to improve the iron status of food is a very inexpensive and creative approach to reduce iron deficiency anemia in developing countries.

Abdulaziz A Adish *et al.*,(1999) undertook a study to investigate the effect of food consumption cooked in both iron and aluminium vessels on the iron status of 407 young Ethiopian children. Hemoglobin analysis after the intervention(12 months) showed a greater change in the hemoglobin concentration of children who consumed food cooked in iron vessels when compared with children who consumed food cooked in aluminium vessels(1.7 [SD 1.5] vs 0.4 [1.0]g/dL mean difference to 12 months; mean difference between two groups 1.3 g/dL [95% Cl 1.1-1.6]). The weight and height assessment of children after 12 months(adjusted for baseline weight and length) showed mean difference between 0.6 cm (95% Cl 0.1-1.0) and 0.1 kg(-0.01 to 0.3).The laboratory study of total and available iron content of Ethiopian food cooked in iron, clay and aluminium vessels showed that total and available iron was highest in the food cooked in iron pots except for legumes which didn't show any differences in their iron content when cooked in different vessels.

Chapter 3

MATERIALS AND METHODOLOGY

Methodology for the formulation of the iron-rich recipe for the prevention of iron deficiency anaemia was designed using the findings analysed from the literature review. Methodology and materials involve information about ingredients, adulteration tests, pre-processing and processing methods. After the development of the product, they were analysed for their iron content, sensory properties, moisture and fibre content and shelf life. Standardised procedures described by AOAC, BIS and FSSAI are used for product quality analysis.

3.0. Materials And Methodology

3.1.MATERIALS:

3.1.1.Ingredients:

The raw ingredients like Niger seeds and garden cress seeds were purchased from the brand ‘vedicine' and remaining ingredients like rice flakes and jaggery were procured from the local wholesale retailer in Mangalore.

Table 1: *Ingredients used for ladoo preparation*

Ingredients	**Amount(in grams)**
Rice flakes	60
Garden cress seeds	10
Niger seeds	10
Jaggery, cane	20
Total composition of ladoo	**100 gm**

3.1.2. Equipment and glassware:

Equipment:

Weighing balance (Essae-Teraoka Pvt. Ltd), Hot air oven (B.D. instrumentation, Ambala Cantt), muffle furnace (Rotek instruments, Kerala), 2375 Double beam spectrophotometer, water bath (Labotech instruments, B.D. instrumentation, Ambala Cantt).

Glassware:

Beakers, Test tubes, Conical flasks, Glass rod, Burette, Standard flask, measuring cylinder, Watch glass, Petri dish, Pipettes, Micropipettes, Conical flask.

3.1.3. Reagents and chemicals:

All reagents and chemicals used in the experiments were of AR grade procured from Fisher Scientific, Mumbai; Medilise, Kerala; Emplura, Mumbai.

- Concentrated Sulphuric acid
- Sodium hydroxide pellets

- Concentrated Hydrochloric acid
- Potassium thiocyanate
- Potassium chloride
- Ammonium per sulphate
- Distilled water
- Double distilled water.

3.2 METHODOLOGY :

3.2.1. Quality assessment of Ingredients

Raw materials like Rice flakes, Niger seeds, Garden cress seeds and Jaggery were tested for the presence of adulterated in them(FSSAI)

Table 2: *Quality analysis by Adulteration test*

Ingredient	**Test**	**Adulterant**
Rice flakes	Take 2-3 grams of sample and place it in a Petri dish and examine visually	Extraneous matters(dust, stone, straw, insects, hair)
Niger seeds	Take 2-3 grams of sample and place it in a Petri dish and examine visually	Extraneous matters(dust, stone, straw, insects, hair)
Garden cress seeds	Take 2-3 grams of sample and place it in a Petri dish and examine visually	Extraneous matters(dust, stone, straw, insects, hair)
Jaggery	Collect 1 gram of melted sample and add a few drops of concentrated hydrochloric acid and mix well.	Washing soda, chalk powder.
	Take 1 gram of melted sample and add 3 ml of alcohol and few drops of concentrated hydrochloric acid. Mix it well.	Metanil yellow colour

3.2.2 Standardisation of Processing methods of product.

3.2.2(a) Roasting:

Rice flakes were subjected to dry roasting for 3 minutes in an iron Kadai.

Niger seeds were subjected to dry roasting for 5 minutes in an iron Kadai.

3.2.2(b) Soaking :

Garden cress seeds were subjected to soaking in plain water in the ratio (1:2) for 3 hours at room temperature.

3.2.3 Different variations of products:

Table 3*: Different formulations of iron-rich ladoo*

Ingredients	**Variation A**	**Variation B**	**Variation C**
Rice flakes	50	60	50
Niger seeds	10	10	20
Garden cress seeds	20	10	10
Jaggery	20	20	20
Total composition of ladoo	100	100	100

3.2.4. Estimation of Iron content by Wong's method(Wong, 1928).

3.2.4 (a) Preparation of Ash solution:

The powdered food sample of 5 grams was taken in the crucible whose weight was noted previously. The crucible was kept in a hot air oven at 100°celsius for 3-4 hours and the content of the crucible was charred by placing them directly on the flame of Bunsen burner. The charred crucible was placed in the muffle furnace which was preheated to 500-600° Celsius for 6-8 hours. The ash content was cooled in the muffle desiccator for 30 minutes. The crucible was placed in the furnace for one more hour and was cooled for 30 minutes in the desiccator. The cooled sample was dissolved with 5 ml of concentrated Hydrochloric acid and a drop of water in the water bath until the solution was completely evaporated. The process was repeated with concentrated Hydrochloric acid and water mixture of 4:1 ratio and then 1:4 ratio. The solution was subjected to boiling for 8 minutes and then was cooled. It was then filtered by Whatman's filter paper into a 100 ml standard flask which was then made up to Mark by adding distilled water. The aliquots were then used for mineral analysis.

3.2.4(b) Wong's Method:

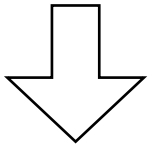
The iron content in the food sample was detected by using 1 ml of ash solution as a test sample. Using Mohr's Salt of concentration 100 microgram/ml, the standard iron solution was prepared and 1-5 ml was used which was made up to 5 ml using distilled water. The blank was prepared using 5 ml of distilled water Ash solution of 1 ml was made up to 5 ml using distilled water. To all the test

tubes, 1 ml of 40% Potassium thiocyanate solution, 1 ml of 30%Sulfuric acid and 1 ml of 7% Potassium persulphate solution was added. The test tubes were kept at room temperature for 10 minutes and absorbance was read at 490 nm.

3.2.5. Preparation of Iron-rich ladoo:

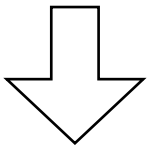
Roasting of rice flakes

Dry roast Rice flakes for 4 minutes and grind them into powder.



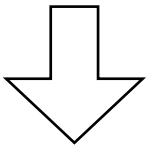
Roasting of Niger seeds

Dry roast Niger seeds, for 5 mins and grind them into powder.



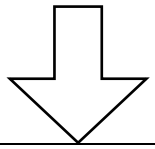
Soaking of Garden cress seeds.

Soak Garden cress seeds for 3 hours



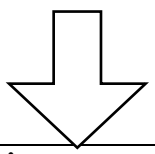
Melting of Jaggery

Melt Jaggery in an iron Kadai by adding water



Mixing

Melted Jaggery is mixed properly with soaked garden cress seeds, roasted Niger seeds and rice flakes



Shaping

Shape into ladoo

The standardised composition of raw ingredients are as depicted in table 1

3.2.6. Sensory Evaluation(By preference Test)

The samples containing the highest amount of iron as per Wong's method were subjected to the preference test with random sampling using the sample size of 50 .

3.3.PROXIMATE ANALYSIS:

Methods described by AOAC 2000 were used for the analysis of moisture and fibre content in the ladoo.

3.3.1 Estimation of moisture content by Hot air oven method:

Iron-rich ladoo samples were powdered and 5 grams of the powdered sample were subjected to drying by hot air oven at 125°celsius for 4 hours. The samples were then subjected to cooling by the desiccator for 30 minutes and the weight was recorded(I_1).

The samples were kept in the oven for 1 more hour at 125°Celsius. The samples were then cooled for 30 minutes in the desiccator and the weight was recorded(I_2). The average of both the weights was calculated and substituted in the formula to obtain the percentage of moisture.

$$\frac{\text{(weight of petridish+sample prior drying)(g) - (weight of petridish+sample prior drying)(g) x100}}{\text{Weight of the food sample (g)}}$$

$$= \frac{\text{(B-C) x 100}}{\text{A}}$$

3.3.2 Estimation of the fibre content

The food sample of 2 grams was weighed in a 500 ml beaker, to which 200 ml of 0.255 N of Sulphuric acid solution was added and boiled for 30 minutes. It was filtered using a muslin cloth and washed with 200 ml of boiling water till all the acid was washed off. The filtrate was transferred to the same beaker to which 200 ml of 0.313 N of Sodium Hydroxide solution was added and boiled for 30 minutes. The filtration of contents was done by filter paper washed with water and was dried for 2 minutes in a hot air oven at

250° Celsius and the dried sample was scraped off to the weighed crucible and was dried again for 100°Celsius for 4 hours. It was then subjected to cooling and weighed, the contents were placed in the preheated muffle furnace at 600°Celsius for 30 minutes, cooled and weighed. The crude fibre content was calculated by the formula:

$$\frac{\textbf{Weight of the crucible before ashing - Weight of the crucible after ashing}}{\textbf{Weight of the food sample(g)}}$$

3.4. SHELF LIFE STUDY OF THE PRODUCT

3.4.1.Sensory evaluation of the food product:

Sensory evaluation of the food product was done by using 9 point hedonic scale rating on the overall acceptability for 1 month period with 4 sessions of analysis on the 1^{st} day, 1^{st} week, 2^{nd} week, 3^{rd} week .

Chapter 4

RESULTS AND DISCUSSION

Iron content analysis by Wong's method showed that three variations of **ladoo** had relatively high iron content and sensory evaluation using preference test showed that variation B ladoo was most preferred when compared with other variations. So variation B iron-rich ladoo was further analysed for its moisture content, fibre content and shelf life.

4.0 Results and discussion

4.1. Adulteration test for the quality assessment of raw ingredients of ladoo.

Table 4: *Adulteration test of raw ingredients***.**

INGREDIENTS	ADULTERANT TESTED	OBSERVATION	INFERENCE
Rice flakes	Extraneous matter (dust, stone, straw, insect, hair).	No extraneous matters were observed .	Rice flakes were not adulterated.
Niger seeds	Extraneous matter (dust, stone, straw, insect, hair).	No extraneous matters were observed.	Niger seeds were not adulterated.
Garden dress seeds	Extraneous matter (dust, stone, straw, insect, hair).	No extraneous matters were observed.	Garden dress seeds were not adulterated.
Jaggery	Metanil yellow	The solution did not turn into blue-red colour.	Jaggery was not adulterated.
	Washing soda and chalk powder	No effervescence was observed.	Jaggery was not adulterated.

From the above table 4, it is understood that raw materials used for the preparation of iron-rich ladoo were of good quality and free from adulterants.

4.2. Standardisation of processing methods.

Processing methods were standardized for better outcomes of the product in terms of its sensory characteristics.

Table 5. *Processing methods:*

Processing methods	Observation	Result
Soaking of Garden dress seeds	• Soaked garden cress seeds were acceptable in terms of their sensory characteristics like colour, appearance, flavour and odour. • Unsoaked garden cress seeds were not acceptable in terms of their sensory characteristics like colour, appearance, flavour and odour	• The soaked garden cress seeds were suitable for Iron-rich ladoo preparation.
Roasting of Niger seeds	• Roasted Niger seeds were acceptable in terms of their sensory characteristics like colour, appearance, flavour and odour. • Unroasted Niger seeds were not acceptable in terms of their sensory characteristics like colour, appearance, flavour and odour	• The roasted Niger seeds were suitable for Iron-rich ladoo preparation.
Roasting of Rice flakes	• Roasted rice flakes were acceptable in terms of their sensory characteristics like colour, appearance,	• The roasted rice flakes were suitable for Iron-rich ladoo preparation.

	flavour and odour. • Unroasted rice flakes were not acceptable in terms of their sensory characteristics like colour, appearance, flavour and odour	

4.3. Iron estimation of different variations of Iron rich-ladoo

Iron content of three different variations of ladoo with different compositions were estimated using Wong's method.

Table 6: *Iron content of different variations of ladoo*

Variations	**Compositions (Total 100 gm of ladoo)**	**Concentration of Iron per gram of sample((g/mL)**
Variation A	• Garden cress Seeds=20 gm • Niger seeds =10 gm • Rice flakes =50gm • Jaggery=20 gm	189.8
Variation B	• Garden cress seeds=10 gm • Niger seeds =10 gm • Rice flakes =60 gm • Jaggery=20 gm	184.3
Variation C	• Garden cress seeds=10 gm • Niger seeds =20 gm • Rice flakes =50 gm • Jaggery=20 gm	198.6

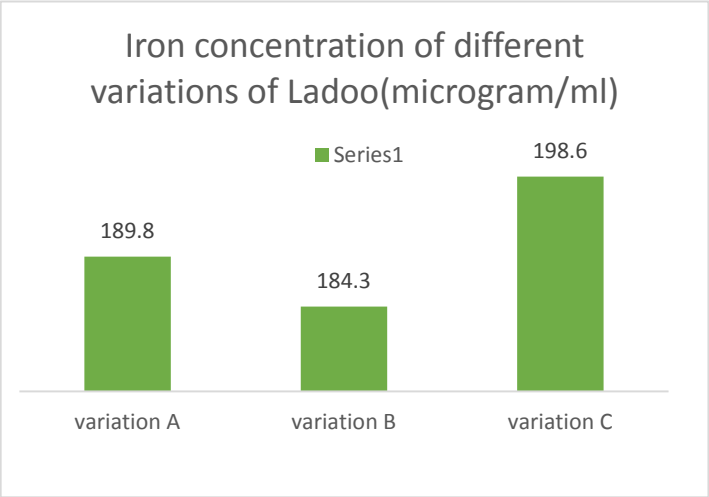


Figure 1:- *The iron concentration of different variations of iron-rich ladoo.*

From **Figure 1,** it is understood that variation C has the highest iron content when compared with the other 2 variations.

4.4. Sensory evaluation using Preference test.

The three variations of ladoo(A, B, C) with different compositions of ingredients were subjected to a preference test to select the final product. The sample size for the preference test was 50. It was observed that out of 50 people, 30 of them liked the variation B ladoo the most, while the remaining 14 people preferred variation A

Figure 2: *Sensory evaluation using Preference test.*

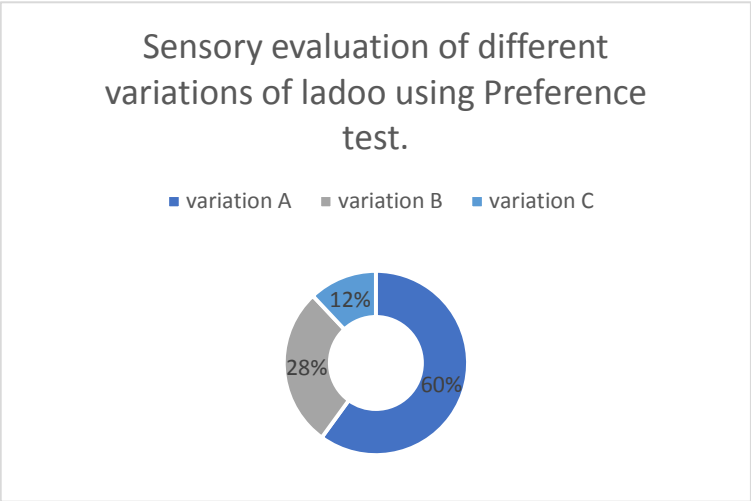


Figure 2 shows the results of the Preference test indicating that variation B ladoo was most preferred in terms of their sensory characteristics like appearance, flavour, colour and odour. So it was selected as the final product.

4.5. Evaluation of final product

Sensory evaluation by preference test showed that variation B ladoo was liked by most of the participants and Wong's test revealed the iron content of ladoo which was high and can be effectively used for controlling iron deficiency anemia.

Figure 3: *Sensory characteristics of the final product*

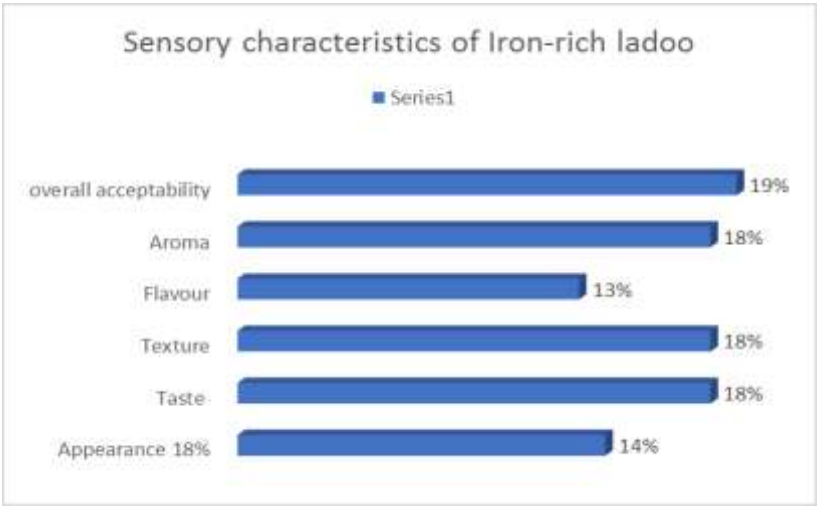


Figure 3. depicts that sensory characteristics of ladoo was acceptable and liked by all.

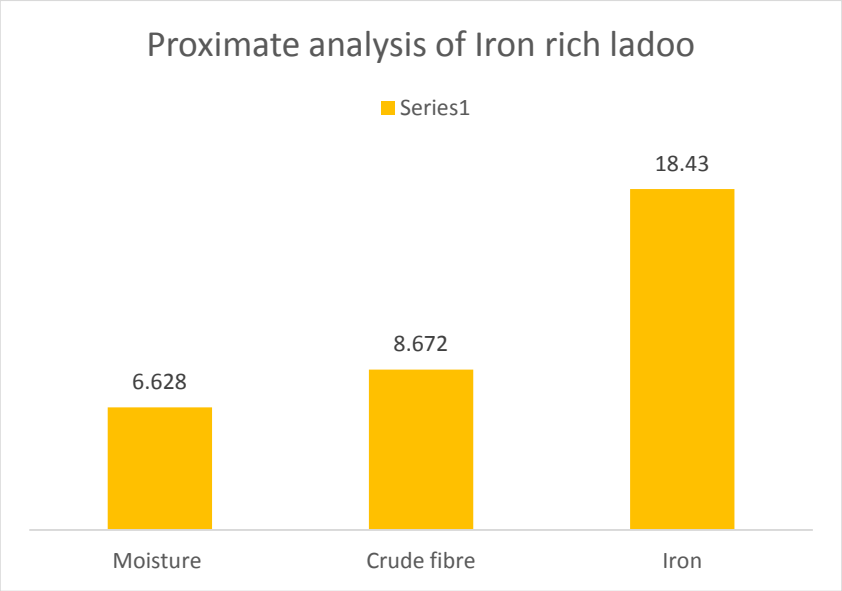
4.6. Proximate analysis

The proximate analysis(moisture, fibre and iron content) of iron-rich ladoo per 100 grams are illustrated in **Table 7** and **Figure 5**

Table 7. Proximate analysis of it on rich ladoo

Particulars	**Composition(100 grams)**
Moisture content	6.628 grams
Crude Fibre	8.672 grams
Total Iron	18.43 mg

Figure 4. Proximate analysis of Iron-rich ladoo.



- Total moisture content reveals the amount of water present in the iron-rich ladoo which was **6.628** grams/100 gram of sample.
- Crude fibre content is the non-digestible component that provides bulk to the diet.

  The crude fibre content of iron-rich ladoo was found to be **8.672** grams/100 gram of sample.
- The total Iron content of e ladoo, which is essential for the prevention of iron deficiency anemia.

  The total Iron content of the ladoo was **18.43** my per 100 gram of sample.

4.7. Shelf life study of the Iron-rich ladoo

Sensory characteristics of the developed iron-rich ladoo were observed in 4 different sessions.

1st day- sensory characteristics of ladoo, i.e. Colour, flavour, taste, appearance and odour were analysed.

1st week- On the 1st week, sensory characteristics of ladoo were acceptable.

2nd week- There was no change in sensory characteristics of ladoo. It was acceptable.

3rd week- There was a noticeable change in colour, flavour and taste was observed indicating its deterioration.

From, the shelf life study it is understood that shelf life of ladoo is **14 days**.

Chapter 5

SUMMARY AND CONCLUSION

5.0 Summary and Conclusion

5.1 Summary

- The adulteration test used for the quality assessment of raw ingredients revealed that all the ingredients used in the ladoo were free from contaminants and were of good quality.
- Processing methods used in the ladoo preparation were dry roasting and soaking which are easy, inexpensive and can be performed at the household level. Roasting and melting of jaggery was done in an iron Kadai in order to enhance their iron content.
- The iron content of the developed ladoo was estimated by Wong's method which was found to be 18.43 mg per 100 grams of sample.
- Sensory evaluation by preference test was used for the selection of the final product from three different variations which showed that variation B ladoo containing 10 grams of garden cress seeds, 10 grams of Niger seeds, 20 grams of jaggery and 60 grams of rice flakes was most preferred and accepted by consumers.
- A sensory evaluation test was conducted to analyse the sensory characteristics of the final product which revealed the final product was highly acceptable in terms of its appearance, taste, texture, flavour and odour.
- Proximate analysis of Iron-rich ladoo claimed that the developed lad had a moisture content of 6.628 gram per 100 gram of sample and fibre content of 8.672 gram per 100 grams of the sample.
- Shelf life analysis showed that the shelf life of the Iron-rich ladoo was 14 days

5.2.Conclusion

Iron is an essential nutrient that is required by our body. Iron is part of haemoglobin, a protein that carries oxygen from our lungs through our body. The deficiency of iron in our body causes iron deficiency anemia, which when left untreated can cause serious health problems. Pre-school children, teenagers and women of reproductive age are the most vulnerable groups at risk of being anemic. So it is important to incorporate iron-rich food in our daily diet to prevent iron deficiency anemia.

An iron- rich ladoo was formulated using iron-rich ingredients like garden cress seeds, Niger seeds, rice flakes and jaggery. Ingredients used are not only rich sources of iron but also provides additional health benefits. Since Ladoo is a sweet snack, it can be easily incorporated into the daily diet of pre-school children and teenagers. So the developed iron-rich ladoo can be effectively used as a nutrient intervention for the prevention of iron deficiency anemia.

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APPENDIX

APPENDIX 1

PREPARATION OF REAGENTS

1. ESTIMATION OF IRON BY WONG'S METHOD

30% sulphuric acid solution

30ml of concentrated sulphuric acid was taken in a 100ml standard flask which was made up to mark with double distilled water.

7% potassium per sulphate solution

7gms of potassium per sulphate crystals were weighed in the analytical balance and were transferred to 100ml standard flask using funnel which was made up to mark with double distilled water.

40% potassium thiocyanate solution

40gms of potassium thiocyanate was weighed in the analytical balance and were transferred to 100ml standard flask using funnel which was made up to mark with double distilled water.

Concentrated potassium permanganate solution

10gms of potassium permanganate was weighed in the analytical balance and transferred through a funnel to the 100ml standard flask and was made up to mark with double distilled water.

Standard iron solution

0.702gms of crystalline ferrous ammonium sulphate (Mohr's salt) was weighed in the analytical balance and dissolved in 100 ml of double distilled water and 50ml of concentrated sulphuric acid and was warmed slightly.2-3 drops of concentrated potassium permanganate solution was added until the solution turned permanent pink colour. The solution was transferred to 1000ml standard flask and made up to with double distilled

water. The concentration of the solution was 1mg/ml. 10 ml of stock solution was taken in a 100ml standard flask which was made up to the mark with double distilled water. The concentration of standard iron solution was 100µg/ml.

2. FIBRE ESTIMATION BY AOAC METHOD.

- **0.255N sulphuric acid solution**

7ml of concentrated sulphuric acid was measured in a volumetric cylinder and was transferred into 1000ml standard flask using funnel, made up to mark with distilled water.

- 0**.313N sodium hydroxide solution**

12.38 Gms of sodium hydroxide pellets were weighed using analytical balance and transferred to a 1000ml standard flask and was added with distilled water until the pellets dissolved. It was made up to mark with distilled water.

APPENDIX 2

QUESTIONNAIRES AND FORMS USED FOR PRODUCT ANALYSIS

FORM 1:

Paired preference test to choose the best ladoo

Name:_____

Date: _____

Note: Rinse your mouth before starting the test. Taste at least half of both the samples A,B and C given, you may drink water after tasting each sample. Re -tasting is not allowed.

Put tick mark on the best sample you liked out of the three on the basis of overall acceptability.

A. ☐ B. ☐ C ☐

Answer these questions considering the ladoo you preferred;

1. Did you like the product?

Yes ☐ No ☐

2.What quality did you like the most?

☐ Taste. Appearance ☐ Texture. ☐

Aroma. ☐ ☐ Flavour

3. Could you make out that the ladoo was made out from Niger seeds and Garden dress seeds?

Yes. ☐ No ☐

If yes, based on what did you recognise_____

4.Do you think that this ladoo would be liked by all age group?

Yes ☐ No ☐

5.Would you like to alter the sweetness of the ladoo?

Increase Sweetness ☐ Decrease Sweetness ☐

Keep It As It Is ☐

6.Would you like it, if, this Iron rich ladoo is incorporated in your daily diet?

Yes. ☐ No ☐

7. On the whole what did you feel about the product?

Very Good. ☐ Good. ☐ Average ☐

Bad ☐ Very bad ☐

FORM 2:

Shelf life Study Of Ladoo Using 9 Point Hedonic Scale Rating

Taste the sample given and rate it from 9 to 1 based on your preference for the particular characteristics mentioned below;

9-Like Extremely

8-Like Very Much

7-Like Moderately

6-Like Slightly,

5-Neither Like nor Dislike

4-Dislike Slightly

3-Dislike Moderately

2-Dislike Very Much

1-Dislike Extremely

Sensory characteristics	**Week1**	**Week2**	**Week3**
Appearance			
Colour			
Texture			
Taste			
Aroma			
Overall acceptability			

APPENDIX 3

OPTICAL DENSITY OF STANDARD SOLUTION USED FOR COLORIMETRIC ANALYSIS

Estimation of iron by Wong's Method

Aliquots of standard solution	**S1**	**S2**	**S3**	**S4**	**S5**	**Blank**
Volume of standard iron solution(ml)	1	2	3	4	5	-
Concentration of standard carbohydrate solution(µg/ml)	100	200	300	400	500	-
Volume of distilled water(ml)	4	3	2	1	-	5
Volume of 30% sulphuric acid solution(ml)	1	1	1	1	1	1
Volume of 7% potassium per sulphate solution(ml)	1	1	1	1	1	1
Volume of 40% potassium thiocyanate solution(ml)	1	1	1	1	1	1
Optical density of standard solution at 490nm						