ASSESSMENT OF MULTIPLE MICRONUTRIENTS IN FORTIFIED FOOD COMMODITIES

(2020-2021)

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B.Sc. FOODS AND NUTRITION

ASSESSMENT OF MULTIPLE MICRONUTRIENTS IN FORTIFIED FOOD COMMODITIES

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BY

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CERTIFICATE

This is to certify that the thesis entitled "assessment of multiple micronutrients in fortified food commodities" is based on the research work carried out independently by Ms. Prachi Patel under the guidance of Prof. (Dr.) Sirimavo Nair in pursuit of a Master's degree of Science (Faculty of Family and Community Sciences) with a major in Foods and Nutrition (Dietetics) and represents her original work.

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Prachi Patel

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ABBREVIATION

BIS- Bureau of Indian Standard

CNNs- Comprehensive National Nutrition Survey

DFS- Double Fortified Salt

EFF-DFS- Encapsulated Ferrous Fumarate- Double Fortified Salt

FNV- False Negative Value

FPV- False Positive Value

FSSAI- Food Safety and Standards Authority of India

GNR- Global Nutrition Report

ICDS-Integrated Child Development Services

IDA- Iron Deficiency Anaemia

IDD- Iodine Deficiency Disorders

IDDCP- Iodine Deficiency Disorders Control Programme

IFA-Iron and Folic Acid

IGN- Iodine Global Network

IT- Iodometric Titration

LBW- Low Birth Weight

MDM- Mid Day Meal;

MI- Micronutrient Initiative

MND- Micronutrient Deficiencies

MNM- Micronutrient Malnutrition

NFHS- National Family Health Survey

NI- Nutrition International

NIN- National Institute of Nutrition

NIPI- National Iron Plus Initiative

NPV- Negative Predictive Value

NSD- National Salt Department

PDS-Public Distribution System

ppm- parts per million

PPV- Positive Predictive Value

QA & QC- Quality Assurance & Quality Control

RDA-Recommended Dietary Intake

TNV-True Negative Value

TPV- True Positive Value

UNICEF- United Nations International Children’s Emergency Fund

USI- Universal Salt Iodization

WHO-World Health Organizations

ABSTRACT

The coexistence of Iron Deficiency Anaemia and Iodine Deficiency Disorders is certain in many developing countries. The most efficient and cost-effective way to address both is to fortify staple food commodities with Iron and Iodine. Looking at the success of universal salt iodization in a country like India, fortifying the common iodized salt with Iron seems to be the most efficient intervention. Salt is the most commonly and regularly consumed condiment despite the economic status, and it is consumed in a fixed small amount daily. This shows the appropriateness of salt as a vehicle for fortification. The development of the technology to fortify iodized salt with Iron has been developed and approved. The standards for the methods have also been published by the FSSAI.

The government has been keen to spare funds to implement this project at large. However, the decision to implement DFS mandatorily is up to the decision taken by the governments of different states. States like Uttar Pradesh, Madhya Pradesh, and Karnataka have implied the production of DFS and its distribution through the PDS and other Schemes.

The large-scale implementation of this project requires continuous Monitoring at each stage of production, transport, sales, and consumption. This calls for strong Quality Control and Quality Assurance. To monitor the quality of DFS, there are gold standard methods (as per the BIS protocol) used as the reference methods. Using these methods in a proper laboratory setting by the skilled professional gives the accurate result for the Iodine and iron content of the DFS. This way, the level of fortification with Iron and Iodine can be monitored and controlled. But these methods require an appropriate environment and trained individuals, which is not suitable for small-scale production units, fields, and consumer levels. To solve this, many quantitative/semi-quantitative analytical devices are made available in the market, which are quite cost-effective plus user- and field-friendly. Still, the validation work for these available devices is yet to be done and published. This study aimed to estimate the Iodine and iron content of DFS samples using the standard methods and spot testing kit. We also aimed to validate thekit against the standard methods.

Our results depicted the adequate amount of Iron and Iodine in the samples using the standard methods (Iodometric Titration and Spectrophotometric Analysis) and the rapid test kit. We found the kit user-friendly and cost-effective for field and small production units. The data showed some variations between the standard readings and the kit readings, which can occur probably due to the temperature or experimental situations. This same work can be repeated with different temperature settings to validate the rapid testing kit in the future.

INTRODUCTION

Iron-deficiency anemia and iodine-deficiency disorders coexist in India; the most cost-effective way to combat both is to fortify staple food commodities available to all population groups with Iron and Iodine simultaneously. Salt is one of the most common and feasible vehicles as every population group consumes it regularly. Therefore, a formula was developed to produce Iron- and iodine-fortified salt (Ranganathan et al., 2007). To implement this strategy at a large scale, the need for continuous monitoring with accuracy rises. This monitoring is to be done at the production by the skilled professionals as well as in the field by the general individuals.

Implying iron fortification needs appropriate QA and QC to ensure optimal levels of Iron in DFS at each stage, i.e., production, distribution, retailing, and consumption. Analysis at a different level of complexity and accuracy to be adapted for different phases of production and distribution systems (Yuan et al., 2008). This calls for an accurate and precise methodology for quantitative analysis at each stage.

For most of the settings, Iodometric Titration *(BIS IS 16232:2014)* for Iodine and Spectrophotometric assay *(BIS IS 16232 – for ferrous fumarate)* for Iron has been accepted as the reference methods. With these accurate standard methods, one can assess the Iodine and iron content of the salt correctly. These methods need a laboratory setting and equipment, as well as skilled personnel to handle the methodology correctly. For this reason, the standard methods cannot be used in the field or in small production units as they lack the laboratory facility and skilled individuals. This leads to the development of the field- and user-friendly quantitative analytical devices with less complexity and accurate results. In the recent past, many devices to assess Iron and Iodine in the salt have been made available. These devices are mostly in the form of qualitative or semi-quantitative rapid test kits, which are easy to use and feasible in terms of fields. One more point to keep in the mind is it is not enough just to verify whether the salt contains Iodine and Iron but rather how much Iodine and Iron it contains is important. So, these quantitative/semi-quantitative kits need to be validated against the standard methods first. For many of these devices, validation reports are published, and for some of them, the validation reports remain unpublished. So, more work is needed for the validation first, and then the comparative analysis is to be done for the devices to assess their user- and field- friendliness. This study was conducted to validate one such analytical device against the standard reference methods.

Malnutrition

Malnutrition, as defined by WHO, refers to the lack or excesses of one or more nutrients either due to intake of nutrients, imbalance of essential nutrients in diet, or impaired absorption of nutrients. Many factors are responsible for malnutrition – Inadequate Diets, Health status, Food Insecurity/Security, Education, Socio-cultural & Behavioural taboos, Social & gender relations, Environmental & Economic Conditions, Political Situations, Technology, and Infrastructure, etc. (Blössner et al., 2005)

All types of malnutrition lead to various forms of illness and different health issues; and are associated with increased mortality rate. According to Global Nutrition Report, we are having *a Double Burden of Malnutrition* globally. This double burden can occur to any population group irrespective of country, city, communities, household, and individual (United Nations, 2018).

One of the major global health challenges the world is facing right now is the Double Burden of Malnutrition. And this broad term covers both undernutrition and overnutrition, as well as some non-communicable diseases related to the diet.

Undernutrition has four broad categories:

1. Wasting – Low weight for height
2. Stunting – Low height for age
3. Underweight – Low weight for age
4. Micronutrient deficiencies – Lack of essential vitamins and minerals in the body

Globally, malnutrition affects people in all countries. Around **1.9 billion** adults are overweight, and **462 million** are underweight. In children, 41 million children under age 5 are overweight or obese, whereas **159 million** are affected by stunting, and **50**

million are affected by wasting**. 528 million** women (29% of all the women) of reproductive age are having Anemia (Global Nutrition Report, 2020).

Micronutrient Malnutrition – Hidden Hunger

Unlike the macronutrients (energy, protein, and fat), micronutrients (vitamins and minerals) are required in small quantities but are vital for our important body functions and physical & mental development. Essential Micronutrients for the human body: Iron, Iodine, Zinc, Calcium, Vitamin A, B-Vitamins, Vitamin C.

Micronutrient deficiencies are the inadequate intake or absorption due to less bioavailability of micronutrients leading to typical ill conditions. These deficiencies cause many health issues such as reduced learning and cognitive ability; impaired growth; reduced immunity; decreased working capacity; several pregnancy complications, blindness, and goitre; and raised risk of mortality. The populations having more risk of hidden hunger are infants and children, women of reproductive age, pregnant women, and the elderly (Faber et al., 2014).

Different from the hunger that is going without food, these deficiencies of micronutrients – Hidden Hunger – many times remain unobserved (FSSAI, n.d.). In contrast to the energy-protein undernourishment, the health impacts of the hidden hunger are not easily visible, but its effects are long-lasting and devastating. Prolonged hidden hunger leads to poor physical and mental health, reduced IQ, vulnerability to infections and diseases, increased mortality in women and children, reduced cognitive development, reduced productivity and physical capacity.

The existence of MNM is very certain in poorer regions of the world as there is undernutrition due to a shortage of food and low dietary diversity. On the other hand, where wealthy population groups can have dietary staples along with micronutrients rich foods (eggs, meat, milk, and milk products) and have access to various fruits and vegetables, poor population groups cannot afford these foods. So, this poor population group relies on more monotonous diets mainly based on cereals, roots, and tubers, and a small amount of those 'rich' foods. This type of diet can provide only a small portion of RDA for most of the micronutrients. Moreover, such groups of people often have a very low intake of fats, which in turn can lead to low absorption of a range of micronutrients across the gut wall. (as fat has an important role in facilitating the absorption). So, a low level of dietary fat may also put such population groups at further risk of micronutrient deficiencies. Same way, these groups consume a few animal source foods; hence they may suffer from a high prevalence of several micronutrient deficiencies concurrently (Lindsay et al., 2006).

More than 2 billion people from across the globe are affected by micronutrient deficiencies, and it is more prevalent in developing countries. Worldwide, there are **three** major forms of micronutrient malnutrition, i.e., Iron, Vitamin A, and Iodine deficiency. Altogether these affect $1/3^{rd}$ of the world's population, the majority of whom are in developing countries. Among these three, Iron deficiency is the most prevalent, and it is estimated that over 2 billion people are suffering from anemia, approximately 1 billion people live at risk of iodine deficiency, and 254 million preschool-aged children are vitamin A deficient (Lindsay et al., 2006).

Iron Deficiency (Anemia), Vitamin A, and Iodine deficiency disorders are major public issues in India. Indian diets are rich in staples, and they provide enough calories but insufficient micronutrients. Consumption of fruits, vegetables, meat, and eggs is considerably low in India. Even though we have a national-level program for supplementation, the results have not been satisfactory. Several micronutrients, including zinc, iron, vitamin A, folate, and Iodine, are the most difficult to satisfy without dietary diversification (FSSAI, n.d.).

Iodine

Iodine is an essential micronutrient for normal brain development. It is vital for the functioning of the Thyroid gland. The activities of thyroid hormones are critical for the normal development of the brain. They increase the proliferation of brain cells and regulate other processes involved in brain function. Thyroid hormone is important for the fourth month of intrauterine life to the third year of postnatal life. Iodine occurs in food as iodide ions or as free inorganic Iodine or in the form of iodine atoms covalently bonded within organic compounds from which they must be freed before absorption. One-third of the Iodine absorbed is taken up by the thyroid gland. For a healthy adult, a daily intake of **150 microgram**s of Iodine is essential. In pregnancy and lactation, the requirement increased up to **200 micrograms**.

Iodine, in its iodide form, is broadly and unevenly distributed on the earth. Leaching from glaciation, floods, and erosions have enriched the surface with iodide, and most of the Iodine is present in the oceans. The cycle of Iodine in many areas is slow and non-complete, which leads to depletion of iodine content in soils and drinking water. So, the population group of these Iodine depleted areas remains Iodine deficient until the addition of the Iodine to foods or the diversification of diets is introduced.

The body of a healthy adult contains 10 to 20 mg of Iodine, of which 70 to 80% is in the thyroid gland. The naturally occurring iodine content of most foods is low. Seafood have a higher iodine content.

RDA for iodine intake:

1. 90 mcg for pre-school children (0 to 59 months)
2. 120 mcg for school children (6 to 12 years)
3. 150 mcg for adolescents (above 12 years) and adults
4. 250 mcg for pregnant and lactating women

Iodine Deficiency Disorders (IDD):

When iodine intake falls below the recommended levels, the thyroid gland may not be further able to synthesize enough amount of thyroid hormone. The resulted low level of thyroid hormones – hypothyroidism – is the major cause responsible for damage to the developing brain and other harmful effects, which are collectively known as iodine deficiency disorders.

IDD refers to all the reverberations of iodine deficiency in a population that can be prevented by constant adequate intake of Iodine. Iodine deficiency is a serious public health issue in most countries, affecting about 740 million individuals, which denotes 13% of the world's population. Iodine deficiency causes a spectrum of diseases called iodine-deficiency disorders (IDD), which include 10oiter, cretinism, spontaneous abortion, stillbirth, congenital disabilities, defects of speech and hearing, squint, and psychomotor defects (Agarwal et al., 2010). During pregnancy, IDD can cause low birth weight (LBW), increased chances of stillbirth, spontaneous abortion, and congenital abnormalities (Nair et al., 2015). Iodine deficiency is the major cause of preventable mental and developmental problems (Diosady & Mannar, 2017). Globally around **1.5 billion** people are at risk of IDD, from which more than **655 million** people are already suffering from IDD. Approximately **1 billion** people live in regions at risk of iodine deficiency. In India, around 150 million people are at risk of IDD, and more than **71 million** people are having goiter and other IDD (Bentley & Griffiths, 2003; Toteja et al., 2006).

Age groups	Health consequences
All age groups	Goiter Increased susceptibility of the thyroid gland
Fetus and Neonates	Abortion Stillbirth Congenital abnormalities Perinatal mortality Infant mortality Endemic cretinism
Children and Adolescents	Impaired mental function Delayed physical development
Adults	Reduced work productivity Hypothyroidism

[Table 1.1 Age Groups and Health Consequences of Iodine Deficiency Disorders]

Iron

Iron is an essential element available in the abundant amount on the earth. In the human body, Iron plays various roles in its different forms, which include a protein-bound form (hemoglobin), heme compounds (hemoglobin, myoglobin), heme enzymes, or non-heme compounds (transferrin, ferritin), etc. Our body requires Iron for the synthesis of oxygen-carrying transport proteins- hemoglobin and myoglobin, for the formation of Iron-containing enzymes involved in different cellular activities. Average adult stores about 1 to 3 g of iron in the body. Almost $2/3^{rd}$ of the body iron is found in the hemoglobin present in circulating erythrocytes, 25% is contained in a voluntarily available iron store, and the remaining 15% is bound to myoglobin present in muscle tissue and in a variety of enzymes involved in the oxidative metabolism and many other cell functions. The Iron in our body is recycled and hence conserved by the body. Since our body requires Iron for various cellular functions, a continuous balance between iron intake, storage, and consumption is to be maintained.

Dietary Iron has mainly two forms: heme and non-heme. The primary source of heme compounds in animal products such as meat, poultry, and fish, whereas non-heme compounds are present in vegetables, fruits, legumes, pulses, and cereals. The bioavailability of heme compounds is higher than that of non-heme compounds. And unlike the non-heme compounds, absorption of heme compounds is not influenced by other dietary factors. But despite the bioavailability, the quantity of non-heme compounds in the diet is more than that of heme compounds in many population groups. Major inhibitors of iron absorption are phytic acid, polyphenols, calcium, peptides.

Iron Deficiency Anaemia

In iron deficiency, the Iron from the stores doesn't get mobilized, and the supply of Iron to the tissues is compromised. When there is insufficiency in the absorption of iron to fulfil the body's requirements, iron deficiency occurs. This insufficient absorption can be due to insufficient iron intake, less bioavailable iron intake, increased needs for Iron, or chronic blood loss. When this situation is extended, it leads to iron deficiency anemia. Iron Deficiency is the most common cause of anemia and is a serious public health issue that influences mental and physical development along with productivity.

Iron Deficiency Anemia is characterized by lower hemoglobin concentration *(less than 11.0 g/dl)*. Major contributory factors responsible for anemia are Blood loss, maternal-fetal bridge of iron deficiency, malaria, dietary intake, and malabsorption. It causes reduced immunity & work capacity, maternal and infant mortality, weakened development in children. In pregnant women, IDA can increase the risk of low birth weight or pre-term baby, prenatal or neonatal mortality, low Iron stores in the newborn, depressed physical activity & fatigue, amplified risk of maternal morbidity (Nair et al.,2015). Iron deficiency anemia during pregnancy is a risk factor for pre-term delivery, low birth weight, and, in initial life, can weaken mental development (Allen, 2000; McLean et al., 2009). Globally **2 billion** people are affected by Iron deficiency, typically in low-income countries. In India, it touches more than *50% of women and 70% of children* (Bentley & Griffiths, 2003; Toteja et al., 2006). According to National Family Health Survey (NFHS) – 4, 53% of pregnant women (15-49yr age group) and 58.5% 0f children (6-59months age group) had anemia. (NFHS-4, 2017)

Fortification of Iodized salt with Iron (DFS)

Double fortification of salt with Iodine and Iron can be a justifiable way to combat Iodine and Iron deficiencies. India's *National Institute of Nutrition* (NIN) has taken the initiative of developing Double Fortified Salt, and NIN has also transferred the technology to iodized salt producers in the nation and provided constant Quality Control support (FSSAI, n.d.). In the DFS formulations, salt is fortified to deliver 100% of one's day-to-day requirement of alimentary Iodine and approximately **30%** of one's RDA for dietary Iron. This equals **10 mg** iron compound per day, or around **3 mg** elemental Iron (Horton et al., 2011).

DFS with supplementary Iron and Iodine has been examined since the 1970s – sponsored by the Micronutrient Initiative, Canada. The Food Engineering Research group at the University of Toronto has made a revolution by stabilizing Iodine and Iron through microencapsulation. The selected iron compound, Ferrous Fumarate, is first ground to match the particle size of salt and then encapsulated with the edible fat.

Fortifying the iodized salt with Iron can provide opportunities to control IDA & IDD. A consistent supply of DFS in the diet can help to enhance the iron status along with the iodine status of school-going children (Nair & Joshi, 2014). The efficiency of DFS in combating IDD and IDA is proven. DFS Providing **3.3mg** Ferrous fumarate per kg of iodized salt led to significant improvements in hemoglobin, ferritin, soluble transferrin receptor, and body iron among women Indian Tea pickers in a period of 9 months (Haas et al., 2014).

In 2009, the *Ministry of Health and Family Welfare* sanctioned the adding of Iron in Double Fortified Salt at **0.8-1.1 ppm (mg/g of salt)**. The Food Safety and Standards Authority of India (FSSAI) has permitted the DFS formulation using EFF as the source of Iron in December 2014. Additionally, FSSAI also published the standards for the double fortification of salt with minimum and maximum levels of fortification with Iodine and Iron in the year 2018.

DFS is a cost-effective intervention. Microencapsulated Ferrous Fumarate matches the salt grain size, color, density and is not observable by the consumer when mixed with iodized salt. The DFS and iodized salt prepared by the dry-mixing process and stored at normal room settings had admirable iodine stability for more than one year (Ranganathan et al., 2007).

The absorption of Iron from cereal-based diets, usually consumed in India, ranges from **0.03 to 0.05** (Hallberg et al. 1974 and Narasinga Rao, 1978). Indian diets comprise almost **20-25 mg Iron/day**, and this, along with double fortified salt, should fulfil the daily requirement of Iron, i.e., 1-1.5 mg for a normal adult man (Narasinga Rao, 1978). DFS delivers 1mg of elemental Iron and 40µg iodine with KI and 30µg with KIO_3 per gm of salt. Ferric orthophosphate (3500 mg/kg) along with sodium hydrogen sulfate (5000 mg/kg) was found acceptable (Narasinga Rao & Vijayasarathy, 1975). The bioavailability of Iron from this double fortified salt, once used in the diet, didn't differ from ferrous sulfate. Bioavailability was not affected on storage under hot, humid situations (Nadiger et al., 1980).

The extent to which DFS improves Iodine and Iron status in the conducted trials is somewhat variable; still, its use is documented to be scaled up in several states in India. (WHO,2012). Efforts to improve consistent usage of DFS are required. Monitoring product quality issues, well-designed awareness drives that confirm behaviour change, and strengthening DFS delivery can aid DFS programs to attain the desired impacts on reducing iron deficiency anemia (Cyriac et al., 2020).

Also, in a large-scale program, the feasibility and efficacy of DFS remain less explored. 2 studies have assessed the efficacy of DFS, emphasizing a gap in the evidence. Both studies were done in Bihar, India. In one program, DFS distribution was done via school feeding programs (Krämer et al., 2020), and the other program used social safety nets and retail markets as delivery platforms (Banerjee et al., 2018). These studies conveyed mixed results on DFS intervention impact, coverage levels were either unreported or low, and data on program execution quality are limited (Cyriac et al., 2020).

Looking at the stability of Iodine and bioavailability of Iron in the Double Fortified salt, it is very important to assess the Iron and Iodine content of the DFS samples on a regular basis to maintain and improve the quality. Based on this rationale, the study is conducted to check the Micronutrients in the dual fortified salt samples to find the efficacy of the DFS provided under the Government programs and at the Market level. For different stages of manufacturing and distribution systems, quality analysis is required. Various accurate and complex analytical methods can be adapted for different phases. In many developing countries, technological conditions need simple and cost-effective methods for the determination of micronutrients at the field level. These needs at different levels are to be satisfied: i) standard quantitative analysis at the regulatory level, ii) semi-quantitative analysis at the production level, iii) qualitative/semi-quantitative analysis at the field or household level is enough to monitor the quality of DFS.

Rapid Test Kits

Fortifying salt with Iodine and Iron requires continuous quantitative and qualitative assessment. It is not enough just to verify whether the salt contains Iron and Iodine or not, but rather, how much amount it contains needs to be assessed. This budding need for quantitative assessment challenges the practicality of qualitative (or semi-quantitative) rapid test kits that are being used broadly, and for that, a wide range of products exist. (Rohner et al., 2015)

Quantitative analytical methods assessing iron and iodine content in salt are vital to monitor the adequacy of salt double-fortification at the level of production, transportation, and consumption.

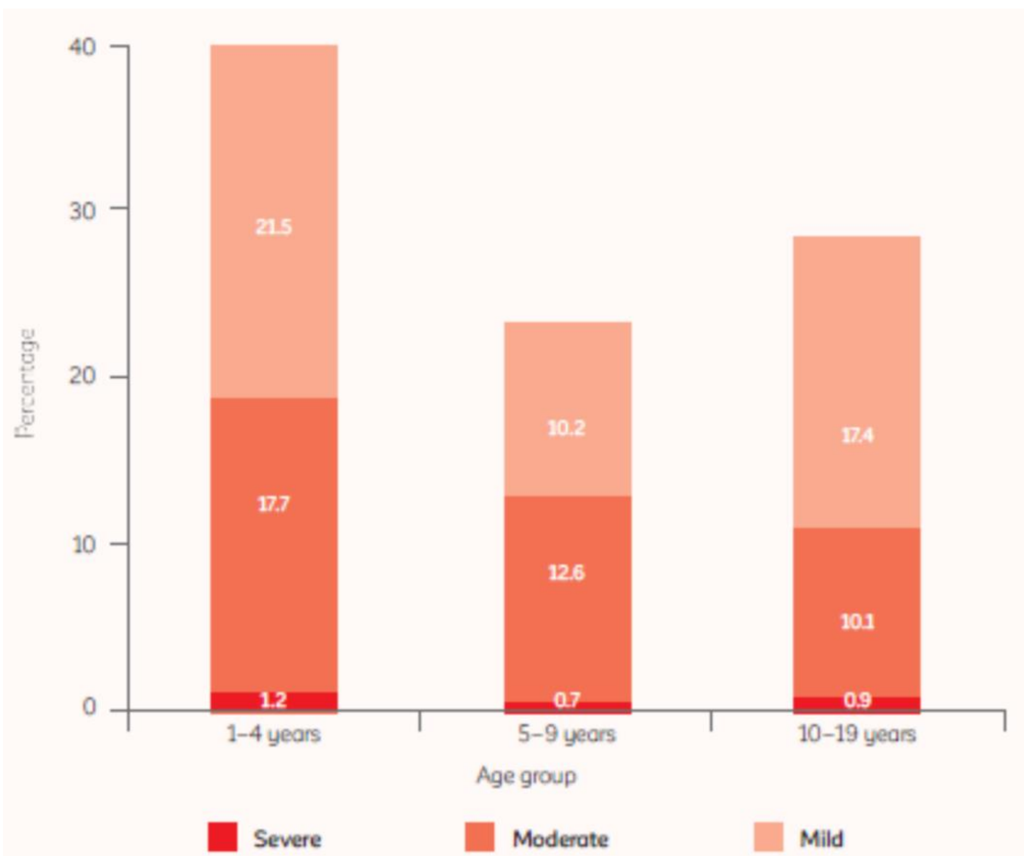
In the recent past, a series of more user- and field-friendly diagnostic devices for quantitative analysis of Iron & Iodine have become accessible. For some of them, methodological validation reports have been published, and for others, only unpublished reports are there. (Rohner et al., 2015) So, more work on these kits' validation is to be done to make it field-friendly without requiring trained personnel. In this way, the assessment at the field level can be made easy. These kits can be advantageous in the quality control of dual fortification of salt in small salt-production facilities and in the fields, predominantly in developing nations. Work on the validation of the currently existing quantitative rapid test kits in a comparative manner has been done by Rohner et al.

Rapid Test Kits are easy-to-use kits that contain small bottles of chemicals required for rapid quantitative analysis of Iodine and iron content of the salt. The method to use is also very simple as we just have to put a small amount of salt on the slide provided, and then adding the reagents in sequence will give us some color which in comparison with the card will tell us the approximate amount.

REVIEW OF LITERATURE

Malnutrition

According to the Global Nutrition Report 2020, Globally, people of all countries are affected by malnutrition. There are about **1.9 billion** adults who are overweight, and **462 million** who are underweight. In children, around 41 million in the age group of under 5 years are overweight or obese, while **159 million** are affected by stunting, and **50 million** are affected by wasting**. 528 million** women (29% of all the women) of reproductive age are having Anemia (Global Nutrition Report, 2020).



[Figure 2.1 Severity of anemia across the three age groups, India, CNNs 2016-18]

In India, anemia is a severe public health issue among all the vulnerable age groups. So India has reviewed and expanded the National Nutritional Anaemia Prophylaxis Programme and included recipients from all age groups, namely children aged 6-59 months, 5-10 years, young people aged 10-19 years, pregnant and lactating women, and women in the reproductive age group under the National Iron Plus Initiative (NIPI) program in 2011. (Kapil et al., 2019)

TABLE 1: IFA SUPPLEMENTATION PROGRAMME AND SERVICE DELIVERY

Age group	Intervention/Dose	Regime	Service delivery
6–60 months	1 ml of IFA syrup containing 20 mg of elemental iron and 100 mcg of folic acid	Biweekly throughout the period 6–60 months of age and de-worming for children 12 months and above.	Through ASHA Inclusion in MCP card
5–10 years	Tablets of 45 mg elemental iron and 400 mcg of folic acid	Weekly throughout the period 5–10 years of age and biannual de-worming	In school through teachers and for out-ofschool children through Anganwadi centre (AWC) Mobilization by ASHA
10–19 years	100 mg elemental iron and 500 mcg of folic acid	Weekly throughout the period 10–19 years of age and biannual de-worming	In school through teachers and for those out-of-school through AWC Mobilization by ASHA
Pregnant and lactating women	100 mg elemental iron and 500 mcg of folic acid	1 tablet daily for 100 days, starting after the first trimester, at 14–16 weeks of gestation. To be repeated for 100 days post-partum.	ANC/ ANM /ASHA Inclusion in MCP card
Women in reproductive age (WRA) group	100 mg elemental iron and 500 mcg of folic acid	Weekly throughout the reproductive period	Through ASHA during house visit for contraceptive distribution

[Table 1.2: IFA supplementation program and service delivery (source: Ministry of Health and Family Welfare, India)]

Solution for Micronutrient Deficiencies

For the control and prevention of MND, WHO has suggested Dietary Diversification (consumption of various food groups including green leafy vegetables and fruits, animal protein, etc.); Supplementation (taking supplements for various micronutrients); Food Fortification of staple foods with vitamins and minerals. Now, Dietary diversification is many times not affordable and not always culturally appropriate for all the groups. Same way, Supplementation is too expensive, and people must remember to take supplements regularly. So, Food Fortification is a more cost-effective and effective way possible to fight MND (Lindsay et al., 2006).

Food fortification

Food fortification is defined as the cautious addition of one or more micronutrients to staple foods, to improve the consumption of micronutrients and to prevent or correct the deficiencies, and to provide health benefits (Lindsay et al., 2006).

Various forms of commercial food fortification programs are there in countries around the world. They include:

1. Mass Fortification [adding of nutrients to foods that are widely consumed by all the groups of the population. It is preferred when most of the population groups are at risk of a particular nutritional deficiency.]
2. Targeted Fortification [when a particular group of people from a population is affected by the risk of nutritional deficiency, this targeted group is provided with the fortified food specifically for them.]
3. Voluntary fortification [when a food company adds nutrients to a food which is not mandated by the government to be fortified.]
4. Mandatary Fortification [when the government issues laws or regulations that require the fortification of certain foods.] (Allen et al.,2006)

Food fortification is technically quite simple for most foods, but to remain justifiable over the long term, there needs to be an active program for monitoring the processes.

According to a 1994 report, the World Bank has demarcated food fortification as: "No other technology offers as large an opportunity to improve lives at such low cost and in such a short time." (World Bank, 1994) Today, hundreds of million people who could advantage from fortified foods are without access to them, whether due to lack of awareness, inaccessibility, poverty, or any such reasons. This must be improved by implementing awareness programs, public distribution facilities for fortified food commodities, government, and political support.

At the Copenhagen Consensus 2008, the Expert Panel ranked food fortification amid the top three international development significances. Precisely, fortification with Iron and Iodine was ranked as a top public health intervention for countries using benefit-cost analysis (Horton et al., 2008).

Garret et al. have done an organized review of 41 reports and 76 research papers and determined that in low and middle-income countries, there is strong evidence of health impact where food fortification attained both high coverage and compliance. The most noticeable advance has been observed in the area of salt iodization. Mean household coverage of iodized salt is 83% in the 52 countries for which there is available data. The number of countries with mandatory salt iodization has progressively increased over time and is now 108. Based on available data on the use of iodized salt, the Iodine Global Network and UNICEF estimate that worldwide over 6 billion people now consume iodized salt. This represents the most noteworthy accomplishment to date of large-scale food fortification. Only 19 countries are still classified with inadequate iodine intake, which is a histrionic shift from 110 countries in 1993. Still, several challenges keep large-scale food fortification from attaining its full public health effect. Various countries with a high level of hidden hunger have not yet started a fortification program. An appropriate selection of food vehicles – those repeatedly consumed by a large part of the population, predominantly the most vulnerable people – coupled with active compliance mechanisms will result in constant growths in the potential effect of fortification programs. (Garret et al., 2018)

Saskia et al. have done a literature review of various publications from 2000 to 2017 pointing nongovernmental organizations whose work emphases on fortification, accompanied by national reports and a "snowball' process of citation searching. The review shows remaining technical challenges, blockades, evidence gaps and prioritizes recommendations and next steps to hasten progress and potential of impact further. This review article has identified and highlighted vital components of successful programs. It also emphasizes issues leading to poor program performance, including inadequate monitoring and execution and poor agreement with standards by industry. In the reviewed 17 years, large-scale food fortification initiatives have been reaching more and more larger parts of populations in low- and middle-income countries. The article recommends that large-scale food fortification and biofortification should be part of other nutrition-specific and nutrition-sensitive efforts to prevent and control micronutrient deficiencies. There are remaining technical and food system challenges, especially in relation to high coverage and quality of delivery and measuring the progress of national programs. (Osendarp et al., 2018)

Miller and Welch (2013) have done a literature review on many food system strategies to prevent the micronutrient malnutrition, one of which is food fortification. In this review, they have studied the effect of farming practices on micronutrients in the food supply. It is also stated that food fortification has the potential to advantage the health status of larger groups of populations. According to the review, salt iodization is the most efficacious large-scale nutritional program to date, and its effectiveness and potential of impact are evident. But the progress in improving the iron status by implementing iron fortification has been slow. The review has concluded that the ideal solution to micronutrient malnutrition is a diverse diet that is safe, nutrient-dense, accessible, and affordable to all population groups. And in addition to this, people must be educated to gain the knowledge to be able to make healthy food choices. (Miller & Welch, 2013)

Das et al. have done a review on micronutrient fortification and its effect on woman and child health. They did a comprehensive exploration to identify available evidence for the impact of fortification. Studies for food fortified with single, double, or multiple micronutrients and the impact of this fortification were analysed using health outcome measures and biochemical indicators of women and children. They have identified 201 studies for the outcomes showing the positive impact of fortification. For children, fortification has an impact on increasing serum micronutrient concentrations. Hematologic indicators were also enhanced, which represents an upsurge when food was fortified with vitamin A, Iron, and multiple micronutrients. For women of reproductive age and pregnant women, the results showed that fortification with Iron has led to a rise in serum ferritin and hemoglobin concentrations. From the observation of 7 studies, they concluded that the iodized salt is effective significantly for increasing the urinary iodine levels. And they also observed ten studies showing the impact of salt fortified with Iron and Iodine on the hemoglobin concentration and on reducing the prevalence of anemia. (Das et al., 2013)

Iron and iodine deficiencies can be prevented through large-scale fortification of principal foods, which are commonly consumed in consistent quantities despite of socio-economic status (Allen et al., 2006; Diosady & Mannar, 2017). In many developing countries, poor rural populations do not buy factory-made foods and have inadequate access to processed staples such as cereal goods, cooking oils, and dairy goods. But they do purchase salt, which is typically centrally administered and purchased, reaching even the poorest rural consumers (Allen et al., 2006; Diosady & Mannar, 2017).

Iodized salt:

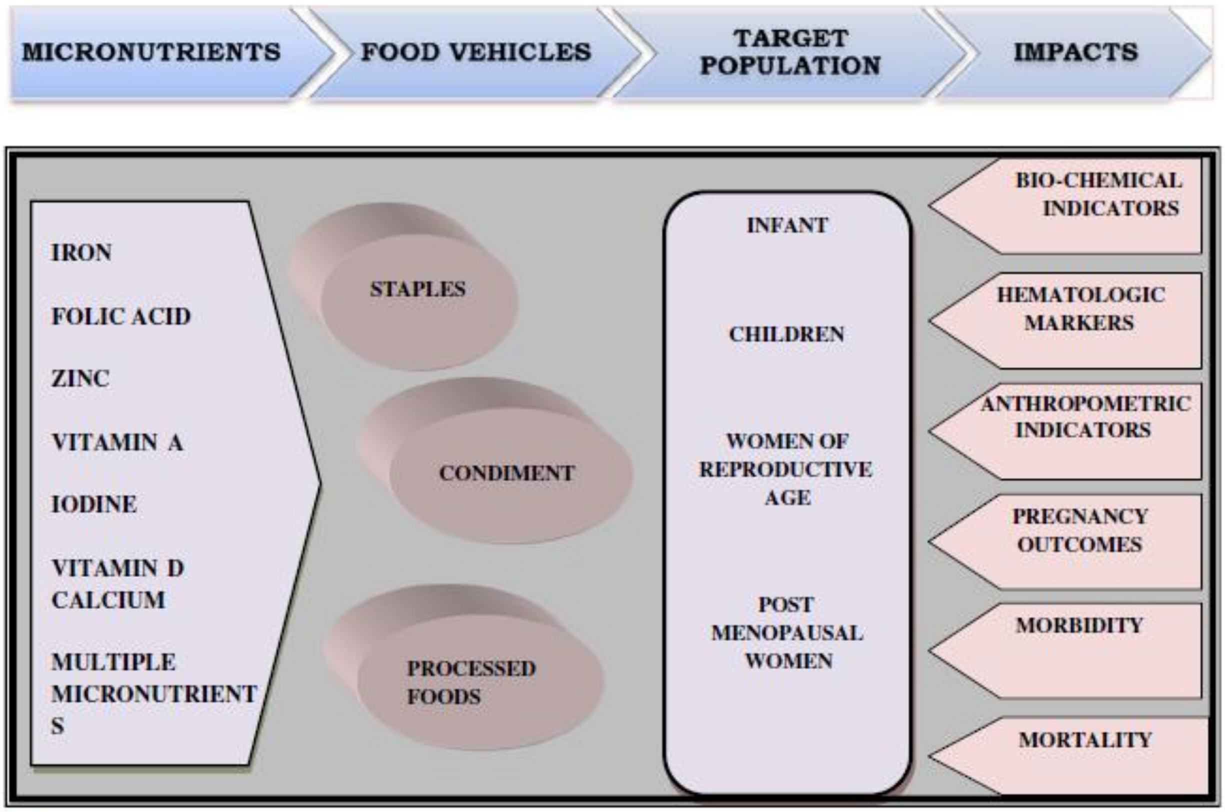
Iodization of salt is administered in 120 plus countries, so most of the world's inhabitants has consistent access to iodized salt (WHO, 2014). Based on available evidence on the use of iodized salt, the Iodine Global Network and UNICEF guesstimate that around **six billion** individuals today consume iodized salt globally. This shows the most substantial accomplishment of large-scale food fortification. Only 19 nations are still left with inadequate iodine intake, and that is a histrionic shift from 110 countries in 1993 (United Nations, 2018). Of the 194 WHO countries, **62%** have optimal iodine intake,

11% having insufficient iodine intake, and **7%** having excess iodine intake, with the **20%** of countries having no data (Iodine Global Network, 2019).

In India, the National policy for Universal salt iodization was made in 1986, and it was made legislated in 2005 at both the countrywide and state level. A survey conducted by *UNICEF & Ministry of Health & Family Welfare* showed that **71%** of the population in the country has access to sufficiently iodized salt and reduction in consumption of non-iodized salt from **34% to 9%**. The production of iodized salt, which was **28.34 lakh tones** during 1992-93, has risen to **68.29. lakh** tonnes in the year 2017-18, which value is closer towards the goal of Universal Salt Iodization (USI) (National Salt Department, 2018). According to National Iodized Salt Intake Survey, the Iodine Deficiency Disorder control program in India is a public health success story, as 92 percent of the population is consuming iodized salt (Yadav & Pandav, 2018).

Salt at the recipients' level (retailer level) should comprise at least a minimum of 15 ppm iodine to deliver a normal RDA of 150 micrograms per day to the consumers. It is generally essential to safeguard the salt has higher Iodine *(30 ppm or more)* at the manufacture level to reimburse for the loss of Iodine in storage and supply.

This give rise to a noteworthy reduction in the prevalence of iodine deficiency diseases. Therefore, it would be easier to use the current iodization set-up to carry Iron along with Iodine through salt to the deficient populations. Addressing iron deficiency through fortified salt has been wished-for since the 1960s (Rao & Vijayasarathy, 1975). But the reactivity and taste of most iron compounds were a challenge in emerging an appropriate iron-fortified salt—iodine and iron interact, which reduces the bioavailability of Iron and leads to sublimation of Iodine.



[Figure 2.2 Conceptual Framework (Das et al., 2013)]

DFS

Diosady et al. have explained the advanced microencapsulation-based technology to produce iron premix for double fortified salt. This premix was found stable and organoleptically indistinguishable when added to the iodized salt. Ferrous Fumarate was extruded first, then cut, sieved to attain a size of 300-710μm (salt grain size). Microencapsulation of the agglomerated extrudates was done to form an iron premix. Microencapsulating process helps to ensure that the added micronutrients are stable without any interaction or degradation. Double fortified salt is produced by blending the iron premix with iodized salt. For the industrial scale-up, this technology was transferred to India. For the establishment and monitoring of the efficient distribution network, Public Distribution system was used. Initially, the scaling-up process was demonstrated in Uttar Pradesh, and after the success, two more Indian states have started the distribution of DFS. According to the study, 60 million people in India are getting the DFS at present. It shows the potential of this health intervention to get scaled up worldwide to ensure a world without iron deficiency anemia. (Diosady et al., 2019a)

METHODS AND MATERIAL

RATIONALE

Implying iron fortification needs appropriate QA and QC to ensure optimal levels of Iron in DFS at each stage, i.e., production, distribution, retailing, and consumption. Analysis at different levels of complexity and accuracy to be adapted for different phases of production and distribution systems (Yuan et al., 2008).

Examining iodine stability provides indirect information about the encapsulation of Iron. If the Iron is improperly encapsulated, unprotected Ferrous ion will react with iodate, which will cause dislocation of Iodine from the iodate, and Iodine is lost by the sublimation process (Krishnaswamy et al., 2019).

Also, the feasibility and efficiency of DFS in a large-scale program remain less discovered. 2 studies have evaluated the efficacy of DFS, underlining a gap in the evidence. Both studies were performed in Bihar, India. In one program, DFS distribution was done via school feeding programs (Krämer et al., 2020), and the other program used social safety nets and retail markets as delivery platforms (Banerjee et al., 2018). These studies conveyed mixed results on DFS intervention impact, coverage levels were either unreported or low, and data on program implementation quality are limited (Cyriac et al., 2020).

Looking at the stability of Iodine and bioavailability of Iron in the Double Fortified salt, it is very important to measure the Iron and Iodine content of the DFS samples on a regular basis to maintain and improve the quality. Based on this rationale, the study is conducted to check the Micronutrients in the dual fortified salt samples to find the efficacy of the DFS provided under the Government programs and at the Market level.

OBJECTIVE

- To assess the Iron and Iodine content of the Double Fortified Salt samples collected from the manufacturers.
- To assess the Iron and Iodine content of fortified wheat flour samples available in the market of Vadodara using the rapid testing kit.
- To check the reliability and validity of the Test Kit against gold standard methods.
- To compare the testing kit results with the gold standard methods.

HYPOTHESIS

Considering a two-tailed test for Iodine and Iron, the estimation may be higher or lower in a rapid test kit compared to the lab-based testing protocol.

SAMPLING

Sampling Method – **Purposive** Sampling (samples obtained from the Multiple Manufacturer)

Sample Size: 114 samples (duplicates/Triplicates) (DFS Samples)

10 samples (duplicates) (Fortified Wheat Flour)

The sensitivity and specificity of the lab-based test will be considered at 90%.

Level of significance: 5% **and Power of test:** 90%.

METHODOLOGY

Sample Preparation and Micronutrient Estimation Procedures for QA & QC (BIS IS 16232:2014) & (BIS IS 16232)

Each of the samples was tested at room temperature and homogenized to ensure uniformity of Iodine and Iron in the given DFS sample.

Following this, the DFS samples were divided into four portions for

a) Testing of Iodine through *iodometric titration (BIS IS 16232:2014)*;
b) testing for Iron through *Spectrophotometer (BIS IS 16232 is for ferrous fumarate)*. The current salt is based on ferrous fumarate, and therefore, we standardized values for Iron in our lab using ferrous fumarate (Sigma Aldrich), (Skoog, Douglas A.; Fundamentals of Analytical Chemistry, 9th ed. 2014) and samples
c) and d) Testing through rapid test kit.

The readings were taken in duplicates. In addition to the internal quality assurance protocol of the vendor, NI provided additional known value samples. One such known value sample was tested after every 10 study samples. For Iodine, in-house reading for the standard was taken after every 10 sample analyses. For Iron, the same procedure was followed every 20 samples. Apart from this, to ensure the quality, all the values were plotted in a standard graph plot. Thus, QA/QC procedure was maintained.

The iodine and iron content of the double fortified salt samples were assessed through *iodometric titration (BIS IS 16232:2014) and Spectrophotometer (BIS IS 16232 is for ferrous fumarate)* methods. The Rapid Test kit method was also used for both Iodine and Iron.

Iodometric Titration Method

Aim: To estimate the concentration of Iodine in the Double Fortified Salt samples.

Chemicals Required:

- Potassium Iodide (KI) (10%)
- Sodium Thiosulphate (2Na2s2o3 5H2O) (0.005M)
- Sulfuric Acid (H_2SO_4) (2N)
- Starch (1%)

Equipment Required:

- Electronic Balance/Physical Balance
- Gas Burner
- Measuring Cylinder
- Pipette- 25 ml
- Burette- 50 ml
- Burette Holder
- Conical flask with stopper
- Glass funnel
- Reagent Bottles
- Wash Bottles
- Glass Stirring Rod

Reagent Preparation:

1. **Potassium Iodide (KI) (10%):**
   Dissolve 100g Potassium Iodide in 1000ml double distilled water. This volume is sufficient for approximately 200 samples. Store the solution in the Refrigerator.
2. **Sodium Thiosulphate ($Na_2S_2O_3$ $5H_2O$) (0.005M):**
   Take 1.24g Sodium Thiosulphate and dissolve it in 1000ml hot double distilled water.
   This volume is sufficient for testing 200 samples. The solution should be stored in a cool and dark place.

3. **Sulfuric Acid (H2SO4) (2N):**

   Pipette 5.56ml concentrated sulfuric acid dropwise into 90ml chilled double distilled water and made the volume up to 100ml.

4. **Starch (1%):**

   Take 1 g soluble starch and make a slurry in 50 ml double distilled water. Add the slurry slowly into 50 ml boiling double distilled water.

Sample Preparation:

Weigh 10g of double fortified salt sample and dissolve it into 50 ml double distilled water.

Procedure:

Add 1 to 2 ml of 2N Sulfuric acid and 5 ml of 10% Potassium Iodide to the salt solution. On shaking it well, the color of the solution will turn yellow. Close the flask with a stopper.

Rest this solution in a dark place for 10 to 15 minutes.

Take the solution out and titrate it against Sodium Thiosulphate until it turns into a pale-yellow color. Subsequently, add a few drops of 1% starch solution. The solution will turn into a dark purple.

Add Sodium Thiosulphate drop by drop from the burette till the solution becomes colorless. Note the final reading. The amount (reading) of sodium thiosulphate in the burette is converted to parts per million using the conversion table.

CONVERSION CHART FOR IODINE IN FORTIFIED SALT (PARTS PER MILLION)

Salt fortified with Iodate or Iodide

Volume Thiosulphate (mL)	Iodine (ppm)	Volume Thiosulphate (mL)	Iodine (ppm)	Volume Thiosulphate (mL)	Iodine (ppm)	Volume Thiosulphate (mL)	Iodine (ppm)	Volume Thiosulphate (mL)	Iodine (ppm)	Volume Thiosulphate (mL)	Iodine (ppm)
0.1	1.1	2.0	21.2	3.9	41.3	5.8	61.5	7.7	81.6	9.6	101.8
0.2	2.1	2.1	22.2	4.0	42.4	5.9	62.5	7.8	82.7	9.7	102.8
0.3	3.2	2.2	23.3	4.1	43.5	6.0	63.6	7.9	83.4	9.8	103.9
0.4	4.2	2.3	24.4	4.2	44.5	6.1	64.7	8.0	84.8	9.9	104.9
0.5	5.3	2.4	25.4	4.3	45.6	6.2	65.7	8.1	85.9	10.0	106.0
0.6	6.4	2.5	26.5	4.4	46.4	6.3	66.8	8.2	86.9	10.1	107.1
0.7	7.4	2.6	27.6	4.5	47.7	6.4	67.8	8.3	88.0	10.2	108.1
0.8	8.5	2.7	28.6	4.6	48.8	6.5	68.9	8.4	89.0	10.3	109.2
0.9	9.4	2.8	29.7	4.7	49.8	6.6	70.0	8.5	90.1	10.4	110.2
1.0	10.6	2.9	30.7	4.8	50.9	6.7	71.0	8.6	91.2	10.5	111.3
1.1	11.7	3.0	31.8	4.9	51.9	6.8	72.1	8.7	92.2	10.6	112.4
1.2	12.2	3.1	32.9	5.0	53.0	6.9	73.1	8.8	93.3	10.7	113.4
1.3	13.8	3.2	33.9	5.1	54.1	7.0	74.2	8.9	94.3	10.8	114.5
1.4	14.8	3.3	35.0	5.2	55.1	7.1	75.3	9.0	95.4	10.9	115.5
1.5	15.9	3.4	36.0	5.3	56.2	7.2	76.3	9.1	96.5	11.0	116.6
1.6	17.0	3.5	37.1	5.4	57.2	7.3	77.4	9.2	97.5	11.1	117.7
1.7	18.0	3.6	38.2	5.5	58.3	7.4	78.4	9.3	98.6	11.2	118.7
1.8	19.1	3.7	39.2	5.6	59.4	7.5	79.5	9.4	99.7	11.3	119.8
1.9	20.1	3.8	40.3	5.7	60.4	7.6	80.6	9.5	100.7	11.4	120.8

[Table 3.1 Conversion chart for Iodine in fortified salt (ppm); Source: (Makhumula et al., 2013)]

Precaution:

1. While preparing sulfuric acid, add acid drop by drop to chilled water to avoid any harmful accidents. Use of hand-gloves is advisable.
2. While pipetting, take the utmost care of accuracy.
3. Use the Double distilled water only.
4. Store the reagents in a properly closed reagent bottle.
5. Use freshly prepared starch solution.
6. While titrating, add the solution drop by drop.

Spectrophotometric Assessment of Iron

Aim: To estimate the concentration of Iron in the double fortified salt samples.

Chemicals Required:

- Ferrous Fumarate (C4H2FeO4)
- Phenanthroline
- Hydroxylamine Hydrochloride
- Hydrochloric acid (HCL)

Equipment Required:

- UV-Vis Spectrophotometer
- Physical Balance
- Magnetic Stirrer
- Cuvettes
- Measuring Cylinder
- Volumetric flask
- Beaker
- Test-tubes
- Micropipette
- Pipette

- Glass rod
- Test-tube stand

Reagent Preparation:

Standard Preparation:

Weigh 0.25g Ferrous Fumarate and dissolve it into 1000ml double distilled water.

For dissolving, first, take the Ferrous Fumarate and add 100ml water, and put the solution on the Magnetic Stirrer. Now add 3 ml of 2N Hydrochloric acid drop by drop to it. And keep the solution on the Magnetic stirrer till Ferrous Fumarate is completely dissolved. Once dissolved, add the double-distilled water and make the volume up to 1000ml.

Pipette out 25ml stalk solution and make the volume up to 250ml with double distilled water.

Phenanthroline:

Add 0.30g Phenanthroline in 100ml water and make the solution

Hydroxylamine Hydrochloride:

Add 10g Hydroxylamine Hydrochloride in 100ml water.

Sample Preparation:

Take 1g of double fortified salt sample and dissolve it into 25ml double distilled water.

Procedure:

Standardization:

1. Prepare six sets of standard solutions of different concentrations, as shown in the table.

Standard solution	Phenanthroline	Hydroxylamine Hydrochloride	Water
0ml	4ml	4ml	22ml
4ml	4ml	4ml	18ml
8ml	4ml	4ml	14ml
12ml	4ml	4ml	10ml
16ml	4ml	4ml	6ml
20ml	4ml	4ml	2ml

2. Rest these for 10 minutes.
3. Switch on the Spectrophotometer half an hour in advance.
4. After the resting period, take the reading in Spectrophotometer at 512nm.
5. Keep the same sets overnight and take the readings the next day.
6. Now make the standard curve (Concentration-Absorbance) from the readings. Find the slope.

Sample Preparation:

Take 1g salt sample and dissolve it into 25ml water. Then pipette out 2.5ml into a clean test tube. Add 0.4ml Phenanthroline and 0.4ml Hydroxylamine Hydrochloride to the solution. Same way prepare one duplicate.

Keep the set overnight and take the readings in Spectrophotometer.

Now find the concentration using the standard curve.

Precaution:

1. Be very careful while pipetting all the solutions.
2. Wash all the glassware very well and dry them in the hot air oven before using.
3. Weigh every chemical very accurately.
4. While preparing reagents, make sure to add water using pipettes.

Rapid Test Kit Assessment

These kits are made to be used in the field and in the lab to give rapid results. This quantitative assessment can give a result for the adequacy of the Iodine and iron content in the salt.

This kit contains two sets of reagents: labelled with blue color (1 to 3) are for iodine detection and labelled with red color (A to C) are for iron detection. These reagents are prepared using double distilled water.

1. The iodine detection set: Solution 1 contains 1 M H_2SO_4, solution 2 contains Pre-calibrated Potassium Iodide, solution 3 contains an Acidified starch solution with a preservative.
2. The iron detection set: solution A contains 1N HCL, solution B contains pre-calibrated Potassium Thiocyanide, and solution C contains pre-calibrated Hydrogen peroxide.
3. White acrylic plate
4. Toothpick (5)
5. Un-iodized control salt
6. Color image chart of four levels of standard representative colors of actual samples of four different concentrations

Method to use the kit:

Iodine detection:

Keep the acrylic plate on a plane surface. Then spread the sample of control salt (with no iodine and Iron) into two sample sets. Add 1 drop each from the solutions 1 to 3 on the first sample spot. Then add 1 drop each from solutions A to C on the second spot. No or little color development shows the integrity of the test solutions.

Once the integrity of solutions is checked, then you can start the test for actual samples.

Spread the salt sample and make two spots. Add one drop from solutions 1 and 2 on the first spot and mix with a toothpick. Immediately add solution 3 containing starch indicator. Then according to the iodate level of the salt, blue to violet color will start developing. Mix it with a toothpick to get uniform color and compare it with the color chart to record the ppm-level.

Iron detection:

Add one drop each from solutions A, B, and C, respectively. Do not mix with a toothpick. It will destroy the developed spots. Then depending on the level of Iron in the salt, the brick color will start developing. Compare the color with the chart and record the ppm-level.

Blue to dark blue-violet color will show ppm level of Iodine 2 ppm to 16 ppm, and brick color will indicate for iron 300ppm to 850ppm.

Precautions:

1. Keep the kit in a cool and dry place.
2. Control salt analysis need not be done every time, but it can be done 3-4 times during monthly use.
3. Toothpicks and test plate are re-usable, and they should be cleaned with water and wiped with tissue after every use.

ETHICAL APPROVAL

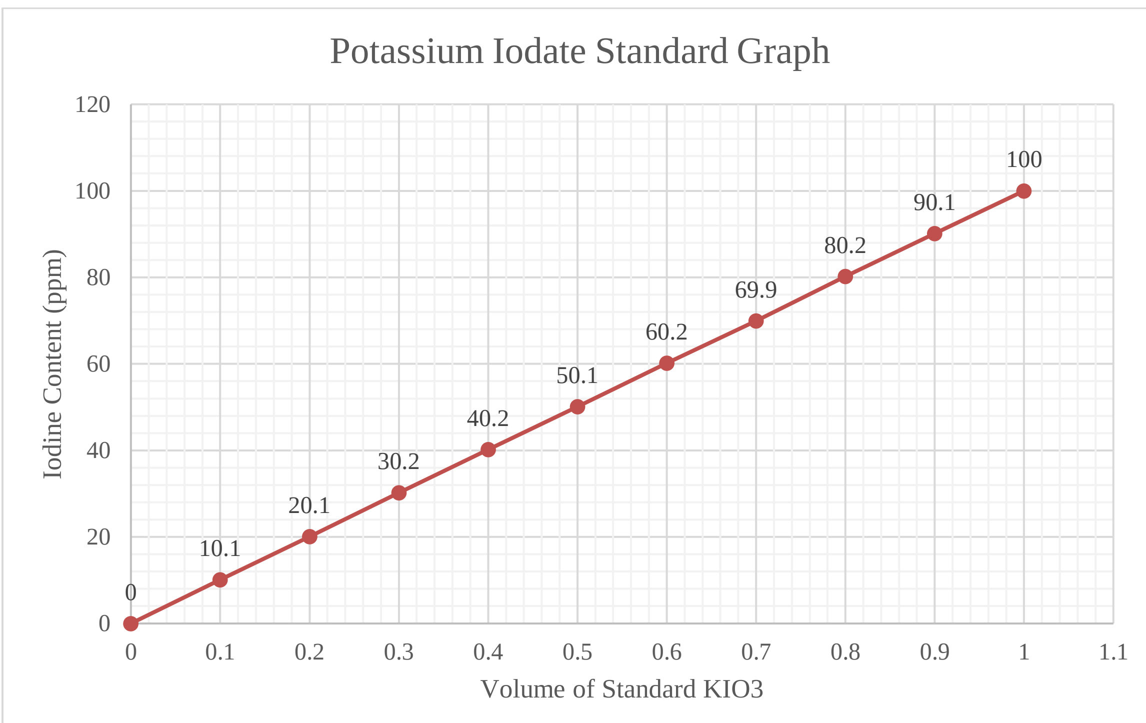
The study was approved by Institution of Medical Ethics Committee and given No. IECHR/FCSC/2020/44, The Maharaja Sayajirao University of Baroda, Vadodara, India.

RESULTS AND DISCUSSION

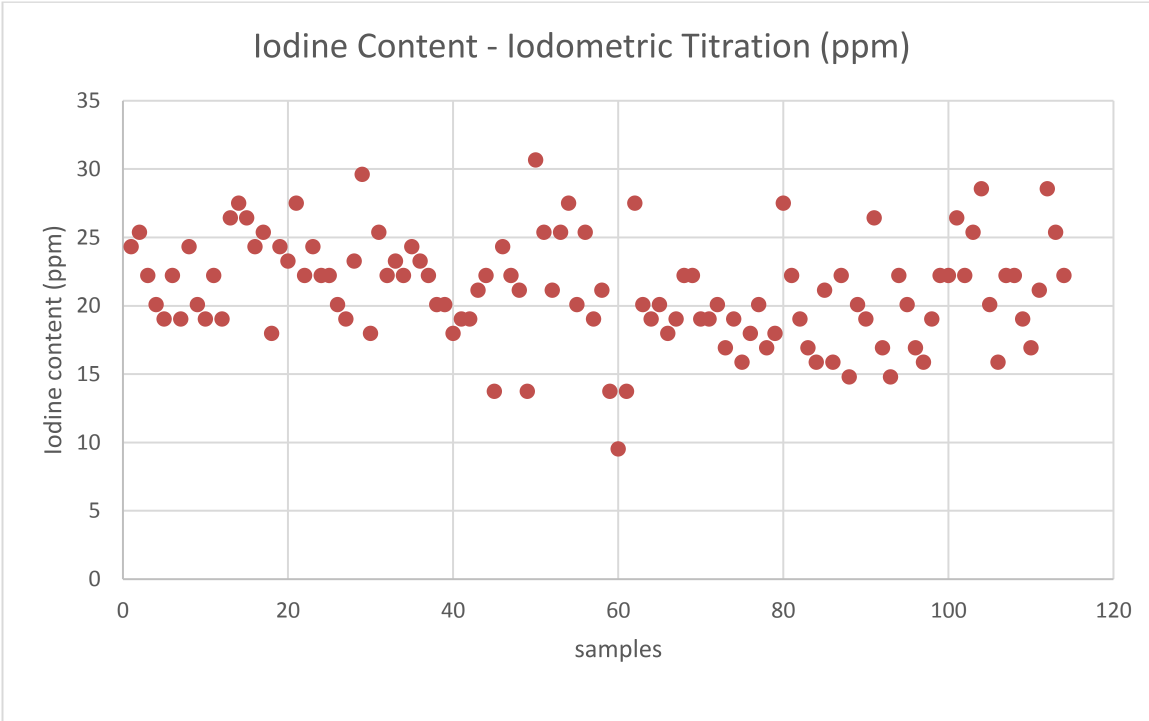
In this study, we estimated the Iodine and iron content of 114 double-fortified salt samples in duplicates. We used standard methods and a rapid test kit for both Iron and Iodine. We conducted the study for 2 main purposes: i) to assess the iron and iodine content of the double fortified salt samples using the standard methods for QA and QC; ii) to assess the iron and iodine content of DFS using Rapid test kit and to validate the kit results against the standard results. We tested each sample in duplicates for both iron and iodine estimation for every method. The methodology was performed in a proper laboratory setting with accuracy. Results showed that there is an adequate amount of Iodine (15-30ppm) present in the DFS sample in proper storage condition.

Iodine estimation – Standard method (Iodometric Titration) (BIS IS 16232:2014)

In the titration method of estimating iodine content, we first standardized KIO_3. Then we titrated salt samples against Sodium Thiosulphate. We used the conversion chart to estimate the iodine content from our burette readings.



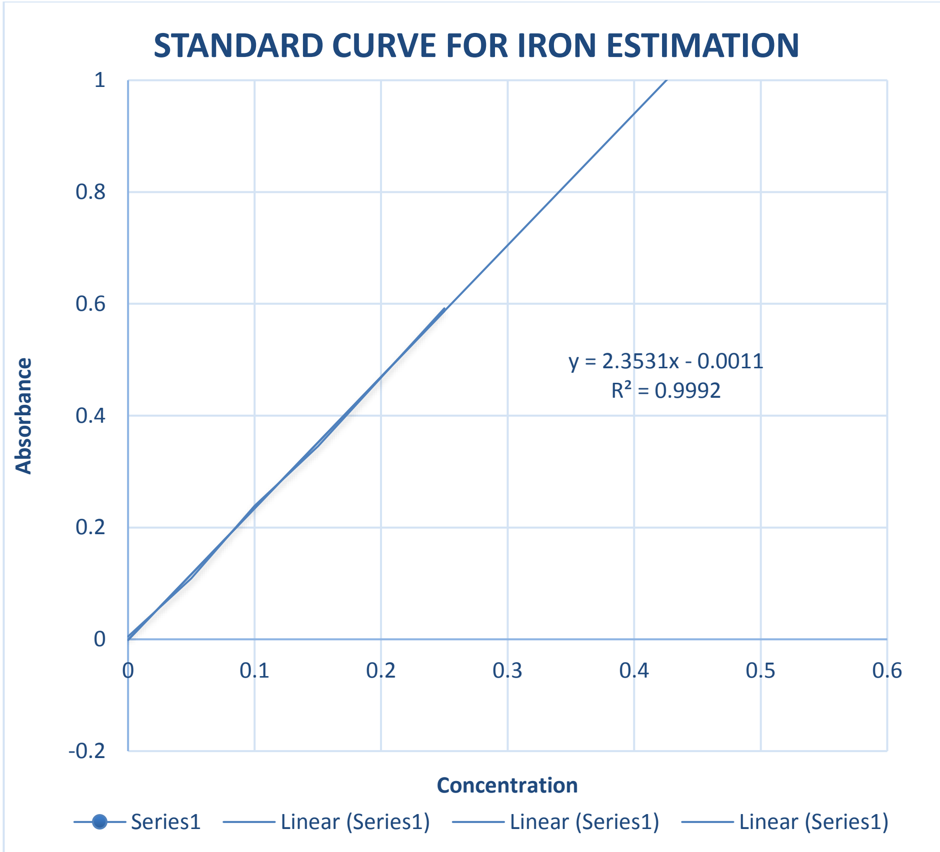
[Figure 4.1 Standardization of KIO3]



[Figure 4.2 Iodine Content – Iodometric Titration (ppm)]

Iron estimation – Standard method (Spectrophotometric analysis) (BIS IS 16232)

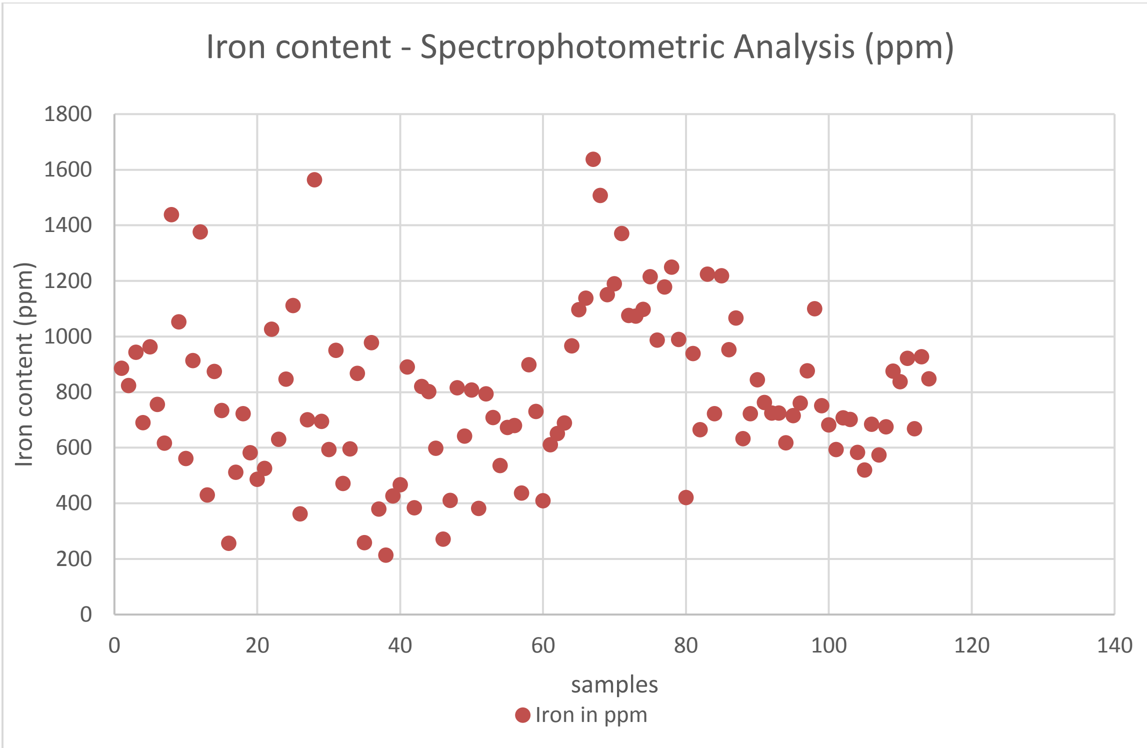
Assessment of Iron in the DFS samples was performed in a well-equipped laboratory setting with utmost care of accuracy. In this method, first, we did the standardization, in which we used Ferrous Fumarate as our standard and made the standard curve shown in the graph below.



[Figure 4.3 Standard curve for iron estimation]

After a few trials, we could achieve the standard curve from which we calculated the slope value. Using this value, it was possible to find the values of Iron in the DFS samples. Once the standard curve was made, we examined the DFS samples in duplicates under the supervision of experts. The result for iron content in the DFS samples showed adequacy in the amount of Iron in most of the samples as per the FSSAI standards (850-1100ppm). While using this method, we could observe that to get the most accurate results from this method; the samples should be prepared and kept overnight in order to get the capsulated Iron released before taking the readings.

Using the Spectrophotometric Analysis, we could observe 72 DFS samples containing an adequate amount of Iron as per the standards (850-1100ppm).



[Figure 4.4 Iron Content – Spectrophotometric Analysis (ppm)]

Rapid Test Kit:

We analysed the iron and iodine content of the DFS samples using the rapid test kit. The results showed the similarities in the values with the standard values.

[Figure 4.5 Comparison between standard method and Rapid Test Kit for DFS samples]

Multiple Micronutrient Assessment

For the multiple micronutrients checking – we carried out a trial for available samples of Fortified Wheat Flour (Atta) to check whether the micronutrients like Iron and Iodine will show reflections of the colours as prescribed by the Kit modality. The following pictures are of the same validation (figure 4.6). The brands of the wheat flour are not disclosed here. Various types of flours have been used for fortification i.e., wheat, rice, maize, rye, etc. As study conducted by Cardoso et al in 2019 elaborated the mandatory processes and regulations implemented on fortification process (Cardoso et al., 2019).

The legislative process serves a purpose to prevent overuse of fortificants and to be best available to the public in its acceptable limits. It also guards against unnecessary food products being fortified, food frauds, etc. thus, it brings benefits to industry, ensures safety, integrity and supply chain management (Esteki et al., 2019). In general, flours and salt are potential vehicles for food fortification since the general public would consume these worldwide.

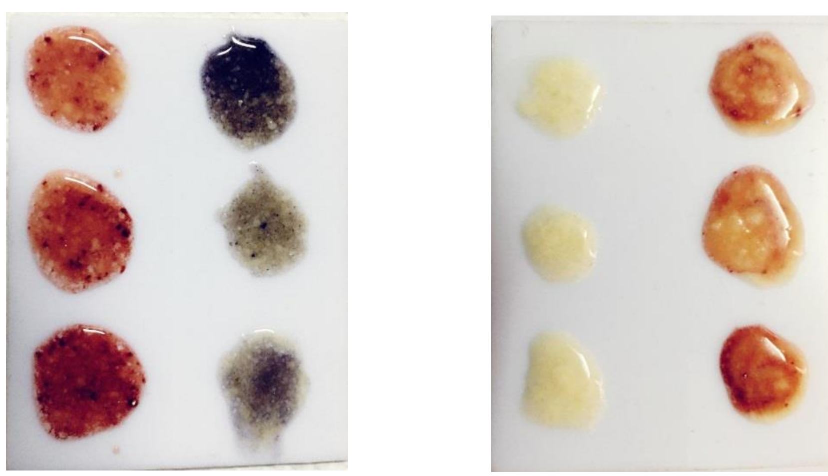
The existing food laws in India have been revisited. The government of India has formulated two new gazettes in the year 2019 and 2202 towards safe foods and food fortification. These are envisaged to bring control on multiple micronutrient malnutrition. In the long run they would benefit the population at large.

We measured the iron and iodine content of the fortified Wheat flour brands available in the Vadodara City using Rapid Test Kit. For this assessment we used three brands available in the market of Vadodara. We estimated the iron and iodine content in the fortified wheat flour in two ways:

1. Fortified Wheat flour with added DFS and

2. Fortified Wheat Flour without DFS.

The result showed the adequate amount of Iron in the wheat flour using the rapid test kit. So, the kit is useful in estimating the iron content of the Fortified Wheat Flour. In the flour samples without DFS, iodine content was negligible whereas samples with DFS showed adequacy in the iodine content. This very work can be repeated in the future as the supply of Fortified Wheat Flour starts on a regular basis.



[Figure 4.6 Assessment of iron and Iodine content of the Fortified Wheat Flour using Rapid Test Kit; the left-side figure shows the result for the Fortified Wheat flour with added DFS and right-side figure shows the result for the Fortified Wheat flour without DFS.]

Validation of Spot Testing Kit method for Iron Estimation

The values from Spectrophotometric Analysis and Spot Testing Kit were categorized into two groups – DFS samples having iron content <850ppm and DFS samples having iron content between 850ppm to 1100ppm.

Iron content using the kit	**Iron content using the standard method**		
	<850ppm	**850-1100ppm**	**Total**
<850ppm	**38** (a)	**26** (b)	**64** (a+b)
850-1100ppm	**21** (c)	**29** (d)	**50** (c+d)
Total	**59** (a+c)	**45** (b+d)	**114** (a+b+c+d)

[Table 4.1 Validation of Rapid Testing Kit]

Here,

A is the True Positive Value – DFS samples having an iron content of 850-1100ppm indicated by both the methods.

B is the False Positive Value – DFS samples having an iron content of <850ppm by the Spectrophotometric Analysis method but 850-1100ppm iron content by the Spot Testing Kit method.

C is the False Negative Value – DFS samples having an iron content of 850-1100ppm indicated by Spectrophotometric Analysis and <850ppm iron content by Spot Testing Kit method.

D is the True Negative Value – DFS samples containing <850ppm iron content according to both the methods.

The sensitivity of the Spot Testing Kit was determined as the percentage of DFS samples having an iron content of 850-1100ppm as identified by the kit method against the samples having 850-1100ppm iron content as assessed by the spectrophotometric analysis method.

Sensitivity = a/a+c*100

Specificity of the Spot Testing Kit was estimated as the DFS samples having iron content >850ppm identified by the spot testing kit against those samples having iron content less than 850ppm as identified by the standard method.

Specificity = d/b+d*100

The Positive Predictive Value was calculated as the percentage of DFS samples with iron content of 850-1100ppm as identified by the standard method, out of those samples identified with iron content of 850-1100ppm by the spot testing kit method.

Positive Predictive value = a/a+b*100

The Negative Predictive Value was estimated as the percentage of DFS samples having less than 850ppm iron content as per the spectrophotometric analysis method, out of those identified to have less than 850ppm iron content as per the kit method.

Negative Predictive Value = d/c+d*100

Specificity, Sensitivity, PPV, and NPV of the Spot Testing Kit for the Iron Estimation

Sensitivity	**64.40**
Specificity	**64.44**
PPV	**59**
NPV	**58**

CONSOLIDATED RESULTS (Iodine and iron Estimation – Standard methods and Rapid Testing Kit) [Table 4.2]

Sr. No.	Pack No.	Iodine content IT (ppm)	Iodine content Kit (ppm)	Iron content SA (ppm)	Iron content Kit (ppm)
1.	**1**	24.32	8	886	850
2.	**2**	25.38	8	824	600
3.	**3**	22.20	4	944	850
4.	**4**	20.09	8	690	600
5.	**5**	19.03	8	963	300
6.	**6**	22.20	8	756	850
7.	**7**	19.03	4	617	850
8.	**8**	24.32	8	1438	600
9.	**9**	20.09	8	1053	850
10.	**10**	19.03	4	561	650
11.	**11**	22.20	8	913	300
12.	**12**	19.03	4	1376	850
13.	**13**	26.43	8	430	100
14.	**14**	27.49	8	874	850
15.	**15**	26.43	8	734	850
16.	**16**	24.32	8	256	850
17.	**17**	25.38	8	512	300
18.	**18**	17.97	4	722	100

19.	**19**	24.32	8	582	600
20.	**20**	23.26	8	486	850
21.	**21**	27.49	8	526	600
22.	**22B**	22.20	8	1026	850
23.	**22**	24.32	8	630	850
24.	**23**	22.20	8	847	850
25.	**24**	22.20	8	1112	850
26.	**24B**	20.09	4	362	600
27.	**25B**	19.03	4	701	600
28.	**25**	23.26	8	1564	600
29.	**26**	29.61	8	695	850
30.	**27**	17.97	4	594	100
31.	**28**	25.38	8	950	850
32.	**29**	22.20	8	471	850
33.	**30**	23.26	8	596	850
34.	**31**	22.20	8	868	600
35.	**32**	24.32	8	259	850
36.	**33**	23.26	8	978	850
37.	**34**	22.20	8	380	600
38.	**35**	20.09	8	214	600
39.	**36**	20.09	8	427	300
40.	**37**	17.97	4	467	600
41.	**38**	19.03	4	890	600

42.	**154.0**	19.03	4	384	600
43.	**156.0**	21.15	8	820	850
44.	**158.0**	22.20	8	802	850
45.	**159.0**	13.74	4	598	300
46.	**162.0**	24.32	8	271	850
47.	**163.0**	22.20	8	411	100
48.	**164.0**	21.15	8	816	100
49.	**165.0**	13.74	2	642	600
50.	**166.0**	30.66	8	808	850
51.	**168.0**	25.38	8	382	600
52.	**169.0**	21.15	8	794	600
53.	**170.0**	25.38	8	709	850
54.	**171.0**	27.49	8	536	850
55.	**172.0**	20.09	8	673	850
56.	**174.0**	25.38	8	680	850
57.	**177.0**	19.05	4	437	600
58.	**178.0**	21.15	8	899	850
59.	**180.0**	13.74	4	730	850
60.	**181.0**	9.51	2	409	100
61.	**182.0**	13.74	4	611	850
62.	**183.0**	27.49	8	651	850
63.	**200.0**	20.09	4	689	600
64.	**220.0**	19.03	4	966	100

65.	**221.0**	20.09	4	1097	850
66.	**222.0**	17.97	4	1138	600
67.	**223.0**	19.03	4	1638	850
68.	**224.0**	22.20	4	1508	600
69.	**225.0**	22.20	4	1151	850
70.	**226.0**	19.03	4	1190	850
71.	**227.0**	19.03	4	1370	600
72.	**228.0**	20.09	4	1076	600
73.	**229.0**	16.92	4	1074	600
74.	**230.0**	19.03	4	1098	600
75.	**231.0**	15.86	2	1215	600
76.	**232.0**	17.97	4	987	850
77.	**233.0**	20.09	4	1178	850
78.	**234.0**	16.92	2	1250	600
79.	**235.0**	17.97	4	990	600
80.	**236.0**	27.49	8	421	850
81.	**237.0**	22.20	8	939	850
82.	**238.0**	19.03	4	665	600
83.	**239.0**	16.92	2	1224	600
84.	**240.0**	15.86	2	722	600
85.	**241.0**	21.15	4	1218	600
86.	**242.0**	15.86	2	953	600
87.	**243.0**	22.20	4	1067	850

88.	**244.0**	14.80	2	633	850
89.	**245.0**	20.09	4	722	850
90.	**246.0**	19.03	4	845	600
91.	**247.0**	26.43	8	763	850
92.	**248.0**	16.92	2	725	600
93.	**249.0**	14.80	2	725	600
94.	**250.0**	22.20	4	618	600
95.	**251.0**	20.09	4	716	850
96.	**252.0**	16.92	2	761	600
97.	**253.0**	15.86	2	877	600
98.	**254.0**	19.03	4	1100	850
99.	**255.0**	22.20	4	751	600
100.	**256.0**	22.20	4	682	600
101.	**257.0**	26.43	8	593	850
102.	**258.0**	22.20	4	707	850
103.	**259.0**	25.38	8	702	850
104.	**260.0**	28.55	8	583	600
105.	**261.0**	20.09	4	520	850
106.	**262.0**	15.86	2	685	600
107.	**263.0**	22.20	4	574	850
108.	**265.0**	22.20	4	675	600
109.	**266.0**	19.03	2	875	600
110.	**267.0**	16.92	4	838	600

111.	**268.0**	21.15	4	922	850
112.	**269.0**	28.55	8	668	850
113.	**270.0**	25.38	4	927	600
114.	**271.0**	22.20	4	848	850

The table values depict the congruency of iron and iodine readings obtained from standard methods (Spectrophotometric Analysis and Iodometric Titration) and from the spot testing kit. However, some variations can also be noted, which could probably be due to the mixing of micronutrients in the salt samples or the experimental conditions. The work suggests the efficiency of spot testing kits in determining the quantity of Iron and Iodine in salt samples through the agreement in readings. However, the study has future scopes of performing the same at different temperatures to validate the usage of kits at any conditions of temperature.

SUMMARY AND CONCLUSION

Governments are ready to use DFS as a public health intervention to address the health and nutritional status of the population and have been willing to spare large-scale funds to procure and distribute DFS through the Public Distribution System at a subsidized rate. The production and distribution of DFS has been promoted by the Government of India as a public health intervention to combat iron and iodine deficiencies.

The Food Safety and Standards Authority of India (FSSAI) has drafted a national technical standard for DFS production. At the state level, the distribution of DFS through PDS is predicated upon by the decision by the state government to include it in the list of commodities offered through fair price shops. The efficacy of DFS has been proven, yet decision-makers did not consider global evidence of the effectiveness of DFS when implying programs. Despite this, in India, the Uttar Pradesh state government decided to conduct its own impact evaluation. Looking at the Uttar Pradesh experiences, states like Madhya Pradesh, Jharkhand, Karnataka have commenced programs to procure DFS and distribute it via their Public Distribution System. Many state governments need to implement this scheme at a large scale in order to reach more and more individuals.

10,000+ frontline health workers who were trained to spread this message are all women who understood the problem and potential of the DFS. Also, the digitalization of the Public Distribution System, which is currently underway, will provide the DFS project with digital data to monitor household-level purchases of DFS and will also allow to spread awareness on the usage of DFS via audio and text messages to the consumers on their mobile phones.

CONCLUSION

In this study, we observed the adequacy of the iron and iodine content in the DFS samples. We found this rapid testing kit easy to use and carry to the field, and it gives rapid results which can be validated against gold standard methods. These and many other available quantitative or semi-quantitative kits need to be validated in the future.

Major Highlights:

- The study was conducted during the period of the COVID-19 Pandemic. This has affected our study situationally.
- The variation in the values between the kit and standard methods can be due to temperature settings or experimental situations.
- The sensitivity and specificity of the kit for iodine estimation could not be calculated due to a wide difference in the values.
- For the Fortified Wheat Flour we assessed only the samples of the brands available in the Vadodara City. We assessed the Micronutrients Iron and Iodine in the wheat flour using the kit. This work still needs future efforts for validation.
- This same study can be repeated with different temperature settings to validate the kit in the future.
- In the study, we could also observe the solubility of Ferrous Fumarate when we used it in our standardization process for iron estimation.

CHALLENGES

Analysis of the DFS project demands for analysing technical feasibility, acceptability & perceptions, and economics.

1. **Technical feasibility** (Organoleptic changes, Technical production, Salt quality)

In many studies, color change of stored salt or cooked foods was observed, which is a significant challenge that directly affects consumer acceptance. The purity, particle size, and moisture content of salt directly affect the quality of DFS to be produced. In India, current specifications for DFS require 98-99% purity of salt; this limits the number of DFS producers. Indian consumers prefer coarse salt, which points toward higher moisture content, larger particle size, and lower purity. Large particle size and high moisture content cause greater loss of Iodine in DFS and a rapid color change to dark yellow regardless of the form of Iron used. So, there's a prerequisite to addressing the threshold whereby moisture content and particle size cause a decrease in Iodine and a change in color.

2. **Acceptability and perceptions:**

The color change was a significant factor when it comes to acceptability; however other factors also created misinterpretations. In India, DFS is a food commodity supplied only through social safety net programs. When it is received through the PDS, it is a part of a predetermined basket of commodities. So, there is no choice given to the shop owner or the consumer when it comes to purchasing DFS, which leads to impressions that the consumer and owner are being forced to buy DFS. This, along with color change, leads to the perception that goods from fair price shops are of inferior quality.

3. **Economics:**

Whether the price is a challenge for the government, producers, and consumers in implementing DFS remains debatable. The government is sparing the funds to make DFS available at a subsidized rate through social safety net programs. The producers are facing issues regarding the cost of production as well as not reaching the retail markets. Consumers are getting DFS only through the PDS, which shows that only a part of the population is buying the DFS that too without any individual choices. This leads to the impression that DFS is of inferior quality.

Many field test kits are available, which gives the rapid result and are user- & field-friendly. The validation of these devices against the standard methods demands more work and publishing. Awareness among the community towards using these devices to increase the uptake by the overall population needs efforts.

[Figure 5.1 Framework of Triple-A approach for Food Fortification by UNICEF, 1997].

Implementing this DFS project on a very large scale requires typical and target-oriented efforts. The Triple-A approach for food fortification given by UNICEF is very useful in this situation. This approach gives the understanding of the actions required for Quality control as well as Quality Assurance.

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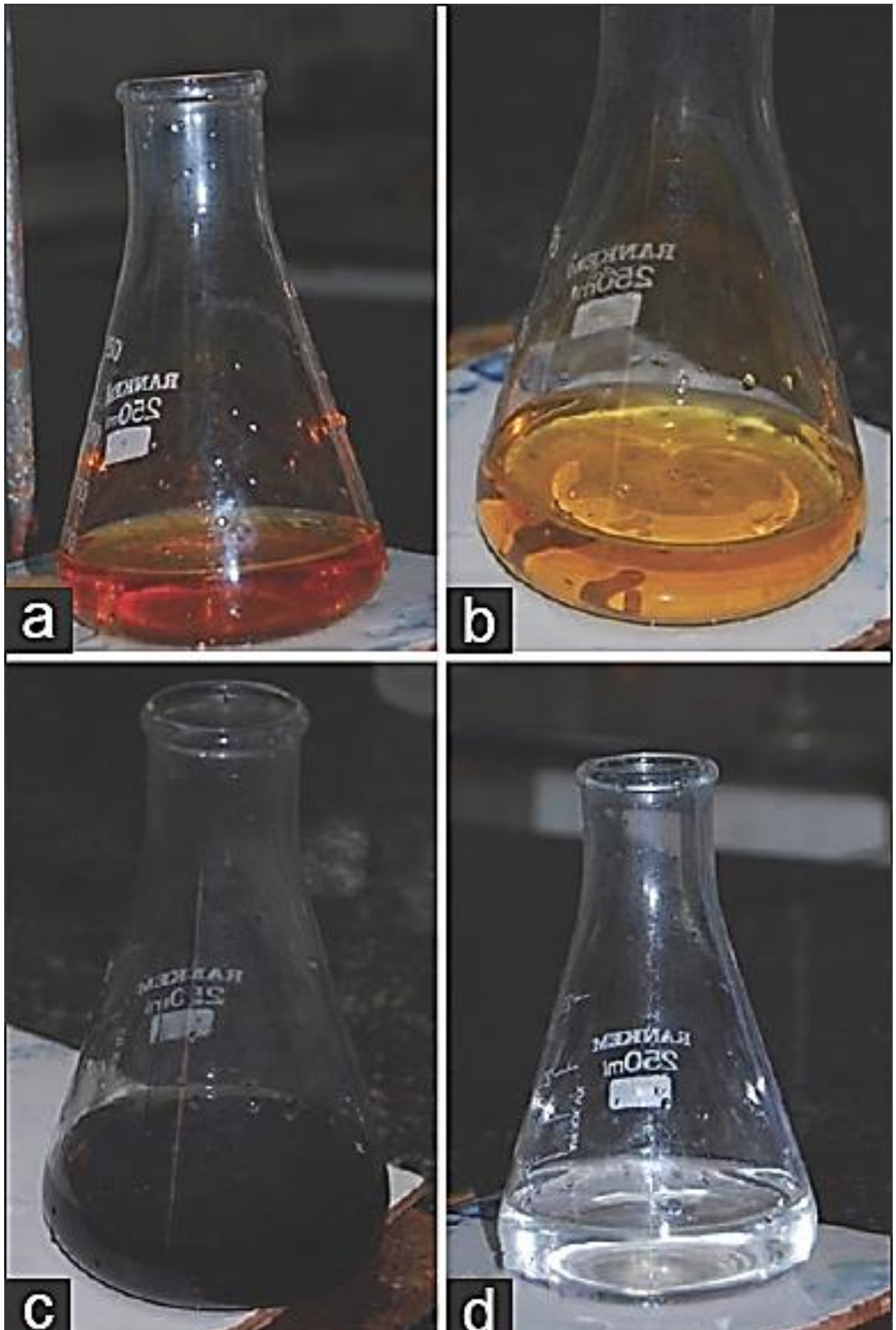
ANNEXURES

ANNEXURE-1

Ethical Clearance Certificate

ANNEXURE-2



Color changes of iodometric titration (a) trituration with sodium thiosulfate (b) medium became light yellow (c) titration stopped by adding starch (d) solution became colorless

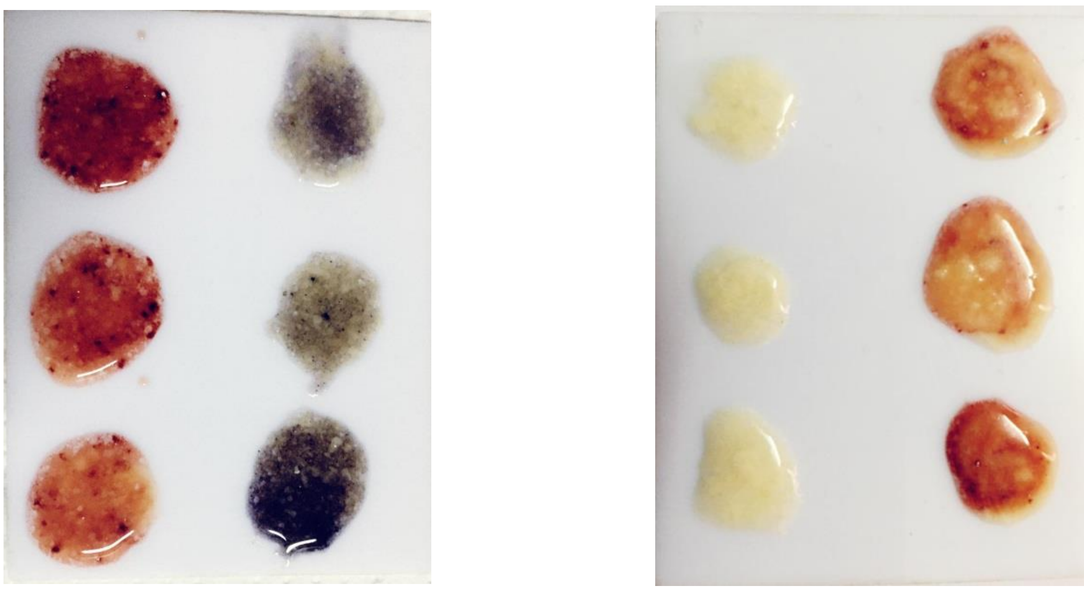
ANNEXURE-3

[Standard Set for Spectrophotometric Assay of Iron]

ANNEXURE-4

[UV-Vis Spectrophotometer]

ANNEXURE-5



(Figure A and B: Analysis of Iron and Iodine content of the Fortified wheat flour using the Rapid Test Kit)

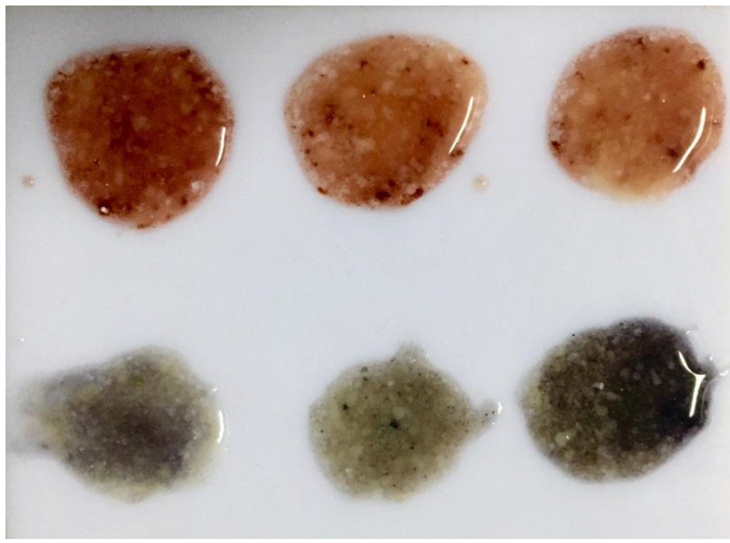
A: Fortified Wheat Flour with DFS added

B: Fortified Wheat Flour without DFS

(From above in the images: 1. Brand a; 2. Brand b; 3. Brand c)

[Rapid Test Kit Analysis of Wheat flour from different brands available in Vadodaara city]

ANNEXURE-6



[Rapid Test Kit Analysis of DFS samples]