INVESTIGATION OF IODINE STABILITY IN SALT DURING DIFFERENT PROCESSING TECHNIQUES AND SPECIFIC TIME INTERVAL

SHARMIN YESMIN SHANTA
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Registration No: 00865
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Department of Applied Food Science and Nutrition
Faculty of Food Science Technology
Chattogram Veterinary and Animal Sciences University
Chattogram - 4225, Bangladesh

December, 2019

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

(Md. Altaf Hossain)
Chairman of the Examination Committee

(Mohammad Shaokat Ali)
Supervisor

Department of Applied Food Science and Nutrition
Faculty of Food Science Technology
Chattogram Veterinary and Animal Sciences University
Chattogram -4225, Bangladesh

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The Author

December, 2019

Dedication

I dedicate this small piece of work to my beloved parents

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List of Abbreviations

Abbreviation	Elaboration
WHO	World Health Organization
%	Percentage
°C	Degree centigrade
Mg	Microgram
Mg	Milligram
mg/L	Milligram per liter
mg/kg	Milligram per kilogram
Gm	Gram
Mm	Micrometer
PPM	Parts Per Million
µg /d	Microgram per day
SD	Standard deviation
Fig	Figure
FAO	Food and Agriculture Organization
T4	Thyroxine
T3	Triiodothyronine
$\mu g\ kg^{-1}$	Microgram per kg
IDD	Iodine deficiency disorder

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Abstract

Iodine is a key regulator of the body's basic metabolic activity and insufficiency of this micronutrient can lead to physical and mental disorder in both adults and children. The main focus of the present study was assessment of iodine in branded iodized salts by applying different processing techniques and specific time interval. In total, 5 salt samples from the market were analyzed and the experiment was done with strict controlled environment in well setup laboratory. The study showed determination of iodine availability and iodine stability of these iodized salts at different conditions. Both titrimetric method and UV-spectrophotometric method were used to determine the iodine concentration in these salt samples. Results revealed that almost all of the salt samples have given positive results of iodine availability. Iodine stability was reduced at the percentage of 7.15%, 8.47%, 9.75%,16.44% and18.04% in normal atmospheric condition whereas in case of boiling, the percentage was 12.95%,13.77% 19.07%, 22.32% and 24.54% for Branded Salt F (BSF), Brand A, Brand Q, Brand C, Brand N respectively after 1st week of opening. However, study findings revealed that during boiling minimum amount of iodine is observed in Brand C and Brand N in last 2 weeks. After keeping the salt samples in environment for third and fourth weeks, Brand F showed greater iodine stability and Brand N iodized salt showed less stability. The study also indicated that iodine loss was maximum during boiling as iodine was exposed to high temperature. On the other hand, loss of iodine was minimum in case of normal atmospheric condition. So, the results depict the loss of iodine after boiling that is why iodized salt should be added after cooking is finished to avoid the loss of iodine. Finally, it is recommended that emergency initiatives should be taken by the government and its related organizations or authority to develop specific awareness on iodine availability and stability of iodized salts.

Keywords: Iodized Salt, Iodine Stability, Universal Salt iodization

Chapter-1: Introduction

Iodine is an important micronutrient which is required for both human and animals. Generally, (10-15) mg of iodine can be found in our body (Prodhan et al., 2014). The availability of iodine depends on iodine intake from foods. Major source of iodine comes from iodized salt, saltwater fish, seaweed, grains and from some medicine. In our body iodine is required for thyroid gland which produces thyroxin (T3) and triiodothyronine (T4) hormone. When human body consumes iodine rich foods, thyroid gland takes this iodine to convert it into thyroxine(T3) and triiodothyronine(T4). Every cell of our body needs thyroid hormone for their metabolism function. Iodine deficiency may cause some disorder during pregnancy like miscarriages, early embryonic death, still births, increased frequency of retained placenta (Kumar, 2003). According to International Council for the Control of Iodine Deficiency Disorders (ICCIDD), and United Nations Internationals Children's Emergency Fund (UNICEF) : daily iodine intake requirement for children is 90μg/d and adults need 150 μg/d and pregnant and lactating mother should intake 250 μg/d (UNICEF-ICCIDD, 2006).

Salt contains higher amount of iodine which is taken by all people within a society amount of potassium iodide or iodate is added during salt iodization. Human body requires very little amount of iodine. Our body cannot produce iodine. On the other hand, Natural food doesn't contain much iodine as human body requirement. For this reason, edible salt is fortified with iodine to fulfill our body requirement of iodine. This can be also used to disinfect drinking water and to prevent surgical infection (Lingvay and Halt, 2012).

Now-a-days iodine deficiency disorder has become major concerning matters in developing countries. Complications have been observed during pregnancy due to iodine deficiency because iodine deficiency impede in fetal development particularly in brain development (Utiger, 1999). According to WHO, In Africa 28.3% people have been suffering from goiter. Salt iodization program has been implemented by approximately 120 countries of the world in 2006. Among these countries, almost 34 countries have become successful to eradicate iodine deficiency disorder by using salt iodization program (United Nations, 2008). Soil contains very little iodine, that's why food needs to be fortified with iodine to overcome this deficiency. An ample percentage

of thyroid hormones ensures normal development of central nervous tissues. So, human body needs iodine for the brain and nervous system for proper function and development (Hetzel and Mertz, 1986).

Thyroid gland releases T3 and T4 hormone into blood stream which bind with protein. Then they start their biological function. T3 becomes biologically abuzz by binding with nuclear T3 receptors which helps to regulate the cellular growth and development (Maberly, 1994). T4 becomes active during pregnancy by binding with protein. Then the deiodinase enzyme converts T4 over free T3 to the fetus. Thus, number of nuclear T3 receptors are highly increased and enhances growth and development of fetus (Ramalingaswami et al., 2000). It becomes highly active in our brain rather than other body part. After performing major development in brain, the whole system gradually becomes slow (Morreale, 2001).

According to WHO, almost 2 billion people were suffering from Iodine deficiency disorder in 1990s; only 20% people received iodized salt. After that Universal Salt Program (USI) was introduced in 1993 to protect people from IIDD. Now it has been adopted in whole world; as a result, almost 95 million infants are being protected every year from difficulties caused by IDD (WHO, 2011). In 1998, IDD among child was decreased in a significant amount due to receiving iodized salt. In the year 2000, USI program was spread to maximum developing countries. Almost 70% people of developing countries adopted fortified salt. In 2011, Due to universal attempt to root out IDD two third of the world population began to use iodized salt (Kiwanis International, 2011).

Bangladesh has major public health problems especially relating to micronutrient deficiencies. Micronutrients are essential for our body. Now iodine deficiency disorder is recognized as one of the major public health problems. Recent study shows that 38% of world's population has been suffering iodine deficiency (Bangladesh Gazette, 1989). Iodine is fundamental micronutrient which is required in minimal amount. Due to iodine deficiency different problem arise in body. But a lot of people are suffering from micronutrient deficiency. Iodine deficiency is one of them. Deficiency of iodine affects at every stage of our life (Okosieme, 2006). Iodine deficiency disorder leads to goiter, premature birth, physical and mental handicap, difficulties in pregnancy etc. Around 1.6 billion people are suffering from this micronutrient deficiency (Mannar and Dunn,

1995). The iodine which we intake from water and food, 90% of iodine comes from food and 10% of iodine comes from water. The amount of iodine which we get from food depends upon the percentage of iodine of the soil in which the crop has been cultivated. It can also vary due to geographical location (Koutras et al., 1985). Though the amount of iodine which we obtain from food is very low, the food is supplemented by salt fortification with iodine more than 100 countries (Mannar and Dunn, 1995).

To prevent iodine deficiency disease, many countries use iodized oil. But the best way to prevent this problem is to use iodized salt. Salt is the best source of iodine. People of all class can easily get iodized salt. During fortification of salt with iodine requires very specific amount of potassium iodide or iodate. This way has been recognized as most cost-effective method. Iodine is not only an excellent supplement; it is also used as medicinal form where iodine is incorporated into multivitamin and antiseptic. Iodine stay in the form of potassium iodide (KIO_3) and potassium iodate (KI). KI cannot be used for a long time because it may degrade easily. On the other hand, for being a stable compound KIO_3 can be used for a long time (Choengchan et al., 2002).

Different methods have been used for the determination of iodine in salt. To determine iodine in salt, Iodometry is the standard method which is used in all over the world. Iodometric titration method is considered one the simplest and cost-effective methods where liberated iodine is titrated with sodium thiosulphate (Tyndall et al., 2013). UV spectrophotometer is also being developed for determining the concentration of iodine by using 352 nm wavelength (Jakmunee and Grudpan, 2001). Beside these methods, some more analytical methods are also being used such as Gas Chromatography-Mass Spectrophotometry (GCMS), electrical method, ion chromatography and x-ray fluorescence. Now a days; Flow injection method (FIA) has been applied for the determination of iodate in normal water by using pirogalol (Ensafi and Chamjangali 2002). Iodine determination also can be done by using other instruments such as ultra-violate spectrophotometer (UV Spectrophotometer), High Performance Liquid Chromatography (HPLC), Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Though Researchers have suggested various methods, some of them are highly costly and need specific instrument (Zhang et al., 2014).

The most widely recommended intervention for eliminating and correcting iodine deficiency disorder is Universal Salt Iodization (USI). Universal Salt Iodization (USI)

was adopted for the prevention of iodine deficiency disorders in 1993. This is also known as a global strategy which declares that salt has to be definitely iodized before making it available for human consumption (Mannar et al., 2009). Bangladesh has also adopted salt iodization program. UNICEF helped initially by providing financial cost. It was agreed to provide 90% expense by giving essential equipment for iodination. UNICEF also provided potassium iodate to all refiners (Patrick, 2008).

Salt iodization was widely accepted rather than other foods because of its availability. On the other hand, salt acts as safe carrier of iodine among other foods. Salt consumption was highly noticeable at a comparatively constant rate (Mannar, 2004). USI strategy along with Govt. and Non-Govt. Organization, salt industry, International traders made the revolutionary transformation in achieving the success (Mannar and Bohac, 2009). The Govt. of Bangladesh also initiated some steps. In 1989, The national assembly made a law for universal salt iodination. It was hoped that iodized salt would be available within 1992 all over the country. The law was modified later and it made a declaration that all marketed salt must be iodized. It was also announced that exchanging and manufacturing of any non-iodized salt punishable crime (Raklrnan, 1982).

1.1 Aims and Specific Objectives

- To evaluate the quantity of iodine present in commercial edible iodized salt.
- To identify the stability of iodine in salt during specific time interval
- To investigate iodine stability by using different processing techniques.
- To compare iodine concentration among different brand salt available in local market.

Chapter 2: Literature Review

An essential dietary micronutrient iodine which is used by thyroid gland to produce thyroid hormone. This thyroid hormone helps to control different functions in our body. It includes proper growth and development of fetus, infants and child (WHO, 2008). This is also essential for normal growth and development brain. It also maintains other basic function including adrenal health, immune function, prevention of cell mutation in human body. when our body becomes unable to produce these hormones, it affects different parts of our body which has become known as iodine deficiency disorder. As a result, this deficiency leads to mental retardation, goiter, growth retardation, reproductive failure and childhood mortality (Patrick, 2008).

2.1 Discovery and Early Investigations of Iodine

The discovery of iodine was made incidentally during the very early part of the 19th century. In early part of 19th century iodine was discovered suddenly. A French chemist named Bernard Courtois discovered iodine during production of gunpowder by sodium salts. He noticed production of purple vapor which was released when sulphuric acid reacts with seaweed (Rosenfeld, 2000). Then another French chemist named Adolphe Chatin discovered that iodine deficiency is related with endemic goiter in 1852 (Chatin, 1852). In the year 1896, Eugen Bauman ensured that iodine is found in thyroid gland (Merke, 1984).

2.2 Iodine

Iodine is a natural chemical element. In 1811, French chemist Bernard Courtois discovered iodine. The element exists as a purple black non-metallic solid at normal temperature. It starts to melt at 114˚C and boils at 184˚C. Iodine-127 is the most stable form of iodine in nature. If it stays in contrast with high energy molecules, it converts to radioactive iodine -129. Iodine is a vital element which is essential for human body. It requires at every stages of human life (Dhaar et al., 2008).

2.3 Source

Iodine can highly be obtained from oceans which is known as reservoir of iodine. On the other hand, the soil of earth contains very little amount of iodine. The distribution of iodine between sea water and land is uneven. Coastal area contains large amount of iodine than the land (Patrick, 2008). The grains which were grown in such land contains very little iodine. Not only grains but also the water in these regions also remain iodine

deficient (Detels et al., 1977). the vegetables which were grown in iodine rich soil contains high amount of iodine (Dhaar and Robbani, 2008).

Table: 2.1 Most Available Dietary Sources of Iodine

Food	Iodine (mcg)
Samon	32
Cod	88
Tuna	27
Shrimp	160
Seaweed	180
Sardine	36
Sea vegetables	250
Yogurt	58
Cheese	22
Soy nuts	41
Eggs	28

Source: (Institute of Medicine, 2006)

2.4 Importance of iodine in human body

All biological functions of human body are assigned to thyroid hormone. Iodine is required for the secretion of thyroxine (T4) and triiodothyronine (T3), which play the vital role for the metabolism of human body. Iodine is required in tiny amount, only 5 gram of iodine is enough to fulfil the life time requirement of an individual having a life span of 70 years (Dhaar and Robbani, 2008). Our thyroid gland stores 70-80 % of iodine. 15-20 microgram of iodine is found in the body of a healthy person. An individual needs total 500 microgram iodine daily; an adult needs 150 micrograms of iodine for physiological demand, 200 micrograms of iodine is required in pregnancy

and lactation period thyroid gland uses 120 mg of iodide for the synthesis of TSH (Detels et al., 1977).

2.4.1 Role of Iodine in Normal Metabolism

Human body needs food to get sufficient energy for completing biological function. Regular metabolism of our body is controlled by thyroid hormone. This normal metabolism of body is impaired (40-60%) due to lack of thyroid hormone. If the body does not get proper amount of iodine which results lack of production of thyroid hormone (Brownstein and David, 2008; Guyton and Hall, 2006).

In pregnancy period, more iodine is required by thyroid gland than normal condition. Iodine performs most authentic role in promoting healthy pregnancy. Iodine plays most crucial role to carry on proper psychological development of fetus. If storage of iodine level falls down below the preconception level during pregnancy, it becomes hard to overcome iodine deficiency disorder. This condition is known as hypothyroxinemia (Smyth, 2006). Miscarriage, neurological disorder, stillbirth these are the common risk which increase during pregnancy due to lack of iodine. Infant becomes physically and mentally stunted because of severe iodine deficiency (Zimmermann, 2009).

2.4.3 Role of Iodine for Growth and Development of Child

Iodine is essential for proper growth and development of children. Lack of iodine leads to retarded physical growth. It has been noticed that those children who reside in endemic area face impaired psychological development (Kochupillai, 1993). Iodine is directly involved in the control of thyrocyte proliferation which is known as autoregulation. The term autoregulation indicates the capabilities of thyroid hormone to rein its proper growth and development (Juvenal et al., 2011).

2.4.4 Role of Iodine as an Antioxidant

Iodine shows the power to boost the antioxidant characteristic in human blood (Winkler et al., 2000). Kupper et al. found that mineral iodine used to inhibit the oxidation process in a sea weed named Laminariales. In this study he found that hydrogen peroxide converted into hypoiodous which is again converted to water instead of forming hydroxyl radical. In fact, during oxidation process excess amount of iodine is absorbed by this alga (Küpper et al., 1998). Lipid peroxidation process was inhibited in Rat's brain by iodine. Brain cells of rats also become less efficient to free radical. Iodine has the capability in boosting the antioxidant property of human blood (Katamine et al, 1985; Cocchi and Venturi, 2000).

2.4.5 Thyroid gland

Iodine is required for thyroid gland to perform those function related to growth and development. T3 and T4 hormones are known as thyroid hormone which are produced from thyroid gland. Metabolism process of iodine is performed by thyroid gland. Thyroid hormones regulate the overall mechanism of our body. These hormones control the body temperature, neurological function, functions of heart, immune system, muscle strength, menstrual cycle, weight gain or loss, breathing etc. (Wisnu, 2008). Everyday almost 80 micrograms of iodine are secreted from thyroid gland in the form of T3 and T4 where 40 micrograms of iodine are secreted from extracellular fluid. Almost, 480 microgram iodine got excreted in urine. Our thyroid gland concentrates highest amount of iodine while the non-hormonal iodine concentrates in mammary glands, eyes, gastric mucosa, salivary gland (Porterfield, 2001).

2.4.6 Iodine and Human Health

By comparing with other nutritional deficiency such as; iron (Fe), zinc (Zn), and vitamin A; iodine deficiency is one of the most common nutritional deficiencies as explained by WHO (Burlingame, 2013). Different research has been studied on Iodine due to its high metabolic importance in human health. Iodine deficiency can be found in different countries of the world. The main reason behind it is the irregular distribution of iodine on the earth's land (FAO, 2009). Amount of required iodine consumption per day exists between (90-200) μg day^{-1} which has been stated by Recommended Daily Allowances (RDA) (RDA, WHO, 2007; Andersson et. al., 2012). It has been estimated that almost 2 billion people intake very low amount of iodine which is responsible for iodine deficiency disorder (Mottiar, 2013). Those diseases which are closely connected with lack of iodine ingestion can be defined as IDD (Zimmermann et al., 2008). The amount of iodine varies from crop to crop which depends on their individual characteristics, origin, preparation, cultivation, handling, processing and storage condition. According to a study, the distribution of iodine in foods is 87 μg kg^{-1}. It was calculated by using geometric mean (Fordyce, 2003).

2.5 Iodine Deficiency Disorders

Our thyroid gland becomes unable to produce adequate amounts of thyroid hormone when ingestion of iodine declines below the recommended range. As a result, presence of low level of thyroid hormone in blood is found which is known as hypothyroidism

and occurs different malfunctions in human body which are recognized as Iodine Deficiency Disorder (IDD) (Hetzel et al., 1983). Recommended level of this hormone is essential for proper development of brain cell. Brain damage, cretinism and decreased cognitive retention occur in severe cases. The most observed result due to lack of iodine deficiency is goiter. On the other hand, the disorder impacts the human life from the fetus to adult with intense health and communal results (Burgess et al., 2007). Thus, the efficiency of the entire society is impaired by IDD. The impact of IDD can also be observed in domestic beast life. The revenue of a country which comes from livestock is also declined intensely (Pandav, 1997).

2.6 Magnitude of IDDs

Lack of iodine content in human body is the most extensive problem in whole world. It has also become the most concerning issue above 130 countries (Swanson and Pearce, 2013). A study found that 33% population of entire world lived in iodine deficient regions in 1996 (Dunn, 1996). At present almost 20 million people are suffering from IDD throughout the world due to ingestion of low amount of iodine (Benoist et al., 2008). According to a study, in India whole population is prone to suffer IDDs due to lack of iodine in their soil where the crops are cultivated (UNICEF, 2009). Lack of ingestion of iodine and insufficient absorption of iodine are the primal factor behind IDDs. People ingest low amount of sea food due to its high cost and inaccessibility. Some food contains goitrogenic element which inhibits the synthesis of thyroid hormone (Hetzel, 1983). Maturation and fast development of all the tissues of human body is retarded due to inadequate iodine content (Patrick, 2008).

2.7 Clinical Diagnosis of Iodine Deficiency and Thyroid Dysfunction

Concentration of urinary iodine, concentration of TSH in blood, amount of free T3, T4 and thyroglobulin (TG) are considered as factors parameters for iodine determination of IDDs. Among these, Urinary iodine excretion has become the most suitable method of iodine deficiency. Presence of Iodine deficiency or excess depends on the urinary iodine concentration (UIC) of a particular population instead of individual person (Jooste and Strydom, 2010).

Table 2.2: Mean Iodine Concentration in Urine (µg/L)

Mean Iodine Concentration (µ/day)	Daily Intake of Iodine (µ/day)	Deficiency Level
Less than 20	Less than 20	Severe Iodine Deficiency
In between 20-49	In between 30-74	Moderate Iodine Deficiency
In between 50-79	In between 149-175	Mild Iodine Deficiency
In between 100-199	In between 150- 299	Optimal level of Iodine
In between 200-299	In between 300-449	Optimal level of Iodine
More than 299	More than 449	Possible excess level of Iodine

Source (National Research Council, 2005)

2.8 Factors Responsible for Iodine Deficiency Disorder

Two primary factors are basically responsible for Iodine Deficiency Disorder. They are Ingestion of low iodine and insufficient iodine utilization. Low iodine ingestion is occurred due to the presence of low iodine in land and it results low iodine content in those grown crops in that area. Beyond this, Sea foods not available to all class of people due to high price. On the contrary, some food contains goitrogens components like glucan which inhibit iodine utilization (Hetzel, 1983).

2.9 Complications of Iodine Deficiency

Iodine is very vital component for development of human growth. Thyroid hormone is synthesized from thyroid gland. Insufficient iodine ingestion results very low production of thyroid hormone which lead to iodine deficiency disorder. The concentration of urinary iodine decreases due to poor production of thyroid hormone (Glinoer, 2007). IDDs cause extreme complication in human body such as loss of fetus brain development, complications in pregnancy period, stillbirth, abortion, abnormalities in behavior of infants, cretinism, mutism, neurological problems, defect in vision, hearing, neuromuscular weakness psychological defects, goiter, mental retardation in adolescent, and increase mortality in child (Boyages, 1993).

2.9.1 Consequences in Pregnancy

An iodine deficient pregnant mother who carries a growing fetus in her womb always stay in high risk. During earlier stage of pregnancy, the thyroid hormone is transferred from mother to fetus which helps to develop the brain of fetus (Groot et al., 2012). But if the mother intakes lack of iodine, she suffers from thyroid dysfunction which causes severe iodine deficiency. It may lead to still birth, abortion and other pregnancy related complexities (Nattero et al., 2019). Gradually that iodine deficient infants suffer from cretinism, impaired growth, reduced brain development and defects in speaking and hearing. The gynecologists need to monitor the pregnant women through proper screening. (Source: Hetzel, 1983).

An early diagnosis and accurate management help to prevent these negative consequences during pregnancy. So, the mother should take proper amount of iodine for the synthesis of thyroxine hormone which is required for the neurological development of the fetus (Taylor and Vaidya, 2016).

2.9.2 Consequences in Neonates

The extreme visible significant of thyroid function is found in Europe between neonates and infants due to lack of iodine. Initial stage of hypothyroidism is highly observed in Europe than in North America (Delange, 1997). This indication is differentiated by postnatally obtained severe hypothyroidism which lasts for a few weeks and needs alternative treatment (Delange et al., 1983).

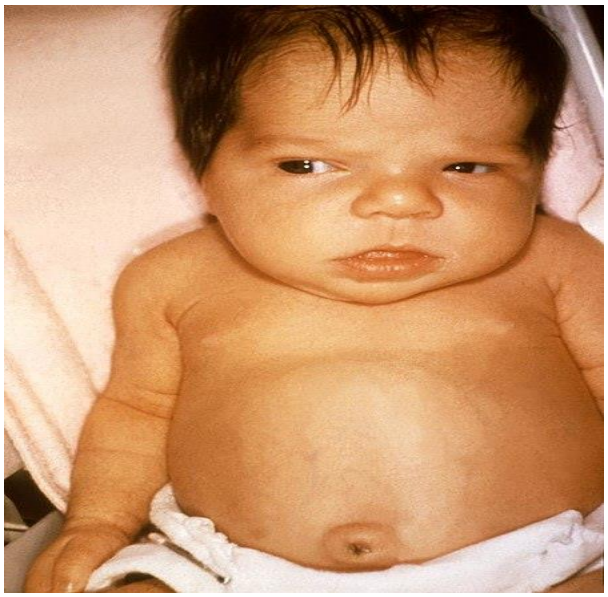

Figure 2.1: 6-week-old female with jaundice due to hypothyroidism.

The chance of transient hypothyroidism rises with the degree of prematurity in neonates Their development of brain depends on sufficient supply of thyroxine. It has been found in goiter endemic region that almost 10 % neonates become at risk of hypothyroidism which cause impaired growth (Delange et al., 1984).

2.9.3 Consequences in Children and Adolescence

Those children who born in endemic area face impaired physical and psychological growth. They also show low level of I.Q. and retarded performance in their institution. Thus, their capacity of learning also reduces gradually due to lack of iodine. Adolescence girls suffer from higher goiter incidence than boys.

In this period, increased pubertal goiter is shown which requires supply of T4 therapy. Metabolism of iodine is increased during this period. Severe iodine deficiency leads to delayed growth in children and hypothyroidism occurs in adolescence (Vir, 2002).

2.9.4 Consequences in Adult

Adults also face iodine deficiency disorder which causes goiter and hypothyroidism in severe cases. Though thyroid activity increases during this period, the percentage of iodine is too low to synthesize thyroid hormone production (Zimmermann et al., 2015). Thus, this long-lasting deficiency of iodine in adults causes goiter hypothyroidism especially in multinodular goiters with autonomous nodules, development of hyperthyroidism, hypothyroidism, apathy, impaired mental growth (Dumont et al., 1995). Autonomous nodules usually occur due to intake of increased iodized salt in diet. So, ingestion of proper amount of iodine is essential to decrease iodine deficiency disorder among adults (Garcia et al., 1999)

Table 2.3: The spectrum of iodine deficiency disorders (IDD)

Physiological Groups	Consequences of Iodine Deficiency
All ages	Goiter Hypothyroidism Increased susceptibility to nuclear radiation
Fetus	Spontaneous abortion Stillbirth Congenital anomalies Perinatal mortality
Neonate	Cretinism mutism spastic diplegia squint hypothyroidism short stature Infant mortality
Childhood	Goiter Impaired learning Stunted growth speech and hearing defects Perinatal mortality Infant mortality
Adolescent	Impaired mental function Delayed physical development Iodine-induced hyperthyroidism
Adults	Impaired mental function Iodine-induced hyperthyroidism

Source: (Hetzel, 1983)

2.10 Present status of iodine in whole world

Almost 38 million children born every year who are suffering from brain damage in developing countries. It has been estimated from a recent research that 266 million school age children intake very low amount of iodine (Benoist et al.,2008). On the other hand, about 2 billion normal population ingest inadequate amount of iodine. In middle east the status of iodine intake varies from country to country. Only Iran and Tunisia have become able to control iodine deficiency disorder. On the other hand, the people of Iraq and Afghanistan were suffering from extreme IDDs. The people of Saudi Arab

suffered moderate level of IDDs. In North Africa, Morocco and Sudan have suffered moderate IDD problems (Azizi and Mehran, 2004). In 1994, WHO suggested to adopt USI programs to secure adequate iodine intake by all people. According to some experts, USI has become the most appreciated public health effort in past 2 decades containing minimum cost to recover IDDs (Mannar et al., 2004). According to UNICEF, iodized salt was used less than 20 % in household of developing countries in the early 1990s but in 2020, this estimation has been increased to 70 %.

Salt iodization program was implemented in almost 120 countries in 2006 (UNICEF, 2008). The scenario has been changed after that. around 12 countries have achieved optimal iodine ingestion on the other hand, sufficient iodine or even excessive level of iodine has been taken in 34 countries (Benoist et al., 2008). In middle east and North Africa, 64% of households have achieved adequate iodine level (Azizi and Mehran, 2004).

2.11 Iodine status in Bangladesh

A survey was commenced entirely in Bangladesh. According to that survey, high level of iodine deficiency has been found all over the country. The Nutrition Survey of East Pakistan 1962-1964 confirmed a goiter rate of 28.9% in former East Pakistan. After the liberation war, The National Iodine Deficiency Disorder Survey 1993 expressed the goiter rate was increased to 47.1% (Rasheed et al., 2001) The Institute of Nutrition and Food Science of the University of Dhaka has also conducted a survey in 1975-76 throughout northern part of Bangladesh including with WHO & UNICEF. The study covered around covered 2,14,608 persons in 417 Upazillas in all 64 districts of the country. Most severely affected districts were Rangpur and Jamalpur having the prevalence rate 27.5% and 29.2%. Another study was administrated in Chakaria upazilla under the Cox's Bazar district of Bangladesh during 1997-1998 to measure the status of iodized salt. A quantitative survey was run to collect information from 21,190 households which expressed that only 1.9% of the household intake iodized salt (Badruddin et al., 2017). Similar study was conducted in Rajshahi city among 500 mothers which declared that the prevalence rate of goiter was 12 %. Those mothers reported that 7% women have never heard about iodized salt and 60% women have heard it from Radio and Television. Open salt was used in maximum household (Boyages et al., 1993). Clinical Another study was carried out among college girls of Dhaka city where 15.3% girls suffer from iodine deficiency. Several pilot projects have

been implemented throughout the country to intake iodized salt. To control IDDs, the National Nutrition Council of Bangladesh organized the first National Workshop in 1981-1982. Deficiency of iodine is endemic in our country. The prevalence rate of goiter was 10.5%. in northern area the rate was almost 30%. On the other hand, the incidence of goiter is less than 5% in southern part of Bangladesh. World Health Organization supervised the full community to find out the main reason behind IDDs. This deficiency occurs due to frequent flood, heavy rainfall and eating habits. People consume low amount of sea fish except in a few coastal areas. 69 % people have low urinary iodine excretion level which was unexpected. The figure has been changed now due to adoption of Universal salt iodination program throughout the country (Yusuf et al., 2008).

2.12 Iodized Salt

Iodized salt was discovered in a grocery shop Michigan, USA in 1924. Salt was added with iodine at 100mg per 1kg was based on the average estimation of 500 µg daily intake. Then some people from other states also tried to make iodized salt till several decades. After that in 1948 U. S Endemic Goiter Committee suggested to introduce mandatory iodized salt to every individual (Kimball and Marine, 1992). Now iodized salt has become available in 90% U.S. household which has been estimated by the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) Global Network. Iodization of salt is very essential to decrease iodine deficiency (Zimmermann et al., 2009). Iodized salt is easily available and the cost is relatively low. Almost 120 countries of the world including Canada and Mexico have also implemented mandatory iodized salt in all types of edible salt (Kimball and Marine, 1992). On the contrary, in USA addition of iodine with salt is optional and FDA does not command to mention the amount of iodine during food packaging. Moreover, the maximum amount of salt consumption appears from processed food. Though the amount of iodized salt is in U.S. is 45mg iodide per kg salt, the recommendation level suggested from FDA is 45 mg iodide per kg salt (WHO, 2007).

2.13 Reason Behind Salt Fortification with Iodine

The aim of food fortification is to decrease the nutritional deficiencies in a community. Many foods were fortified with iodine such as bread, milk, water and salt in last century (Phillips, 1997). Among them salt is most highly selected for fortification.it was first started in USA and then Switzerland (Marine and Kimball, 1920). Gradually universal

salt iodization program became popular all over the world. USI became popular due to the following reasons:

i) Very few commodities are as consumed as salt.

ii) Consumption of salt is constant all over the year.

iii) Only a few specific producers are related to salt business.

iv) The technology of salt iodization is easy to adopt which is also available.

v) The fortification of iodine to salt never affects its taste or color.

vi) The tests to check quality can be monitored at any level of processing steps.

vii) The consumer acceptability of iodized salt is higher than other products.

viii) It takes a very low cost for fortification purpose

So, it can be said that the iodization of salt is very easy to implement. Addition of 20 to 40 parts per million (ppm) of iodine to salt has been recommended to fulfil the iodine requirement of a community (WHO, 1996). Iodine can be fortified to salt by using both potassium iodate and potassium iodide. Iodate is more preferable to iodide because it is more stable under severe climatic condition (humid and hot climates). Though maximum countries of the world use potassium iodate, Europeans and north Americans use potassium iodide (Marine and Kimball, 1920).

2.14 Universal Salt Iodization (USI)

USI indicates adequate amount of iodized salt which is required for all human being has been successfully adopted in many countries. It has been declared as the most hopeful, sustainable, cost effective and environment friendly solution of IDDs (Mannar et al., 2004). This strategy was adopted to ensure adequate intake of iodine by all individuals which has been recommended by the WHO and UNICEF (WHO, 2007). When 90% household of a country intake proper amount of iodized salt (15ppm) thus it becomes successful to implement USI (Global Network, 2009). Massive international

effort has been adopted to implement USI over the last two decades. Consequently, 34 countries have wiped out IDD by adopting USI. A survey has estimated that 70% household of the world consume sufficient iodized salt. In 2006, UNICEF marked 16 countries who needed extra support to extend their efforts to USI. These countries produce very low amount of salt, as a result many unprotected newborns were born. According to UNICEF, the global amount to sufficiently iodized salt might reach to 85 % if these countries achieved USI. Among these countries the name of India was at top position.in 2006, 51% of household consumed sufficient amount of iodized salt and 13 million newborns became unprotected due to IDDs (UNICEF, 2008). In 2013, Around 200 million people were at risk of IDDs and another 71million were suffering from goiter and other IDDs in India. Those countries who have failed to gain 20% iodine coverage suffer from various health difficulties (WHO, 2007). The risks may severely increase during pregnancy if the mother becomes unable to meet required iodine in this period. In this situation, iodine supplementation is considered essential for both pregnant mother and children less than 2year age needs to take oral dose of supplemented iodine (Andersson et al., 2012).

2.15 Iodine in Supplements and Multivitamins

Severe iodine deficiency causes maternal hypothyroidism on neurological development of fetus and neonatal. Everyone is now giving extra emphasis on the adverse effect of mild to moderate iodine deficiency during pregnancy and lactation period of women (Becker et al., 2006). In north America pregnant and lactating mother take daily a supplement which contain 150 μg iodine in 2006 suggested by The American Thyroid Association (ATA).After 5 year they again updated the recommendation and they suggested to ensure 150 μg iodine in any supplement (kelp or seaweed) also in preconception period (Stagnaro et al., 2011). Obican et al. declared that IDD is one of most important preventable micronutrient deficiencies around the world and more than 2 billion people are at risk due to insufficient iodine nutrition. In this case most penetrable groups are pregnant and lactating mother and their developing fetuses (Obican et al., 2012). According to WHO 250 μg of total iodine ingestion should be ensured daily during pregnancy and lactation (WHO, 2007) .On the other hand ,220 μg iodine should intake daily during pregnancy and 290 μg during lactation, more than the 150 μg needs to be taken by non-pregnant adults which have been recommended by and the U.S. Institute of Medicine (Otten et al., 2006).

2.16 Factors which Reduce Iodine Content in Salt

During production specific amount of iodine is added with salt.Accurate amount of potassium iodate or potassium iodide is added during salt iodization process.These can be added as powder form or an aqueous solution or as a dry solid at the stage of production(Manner and Dunn, 1995) . Each country should abide by those specific regulations in every stage of production which have been recommended by the authority.The stability of iodine in salt is an important issue because it directly impact on human health.The actual amount of iodine in iodized salt gradually reduces due to the fallowing reasons :

- Insufficient of iodine added during iodization process;
- Improper distribution of iodine in iodized salt within batches or individual bags;
- Inadequate mixing of salts after the salt iodization process;
- Variation in particle size of salt crystals in a batch or bag;
- Percentage of iodine loss due to the presence of impurities;
- Variation in environmental conditions during storage and distribution;
- Dimunition of iodine during every stage of production (Frorm washing to storage);
- The accessibility of non-iodized salt in different markets;
- The dosclousure of iodized salt to heat and light;
- Expousure of iodized salt in household;
- Using Very poor packaging materials;

(Source : Allen et al ., 2006)

2.17 Methods used to determine iodine

Various methods have been developed to determine iodine in urine, milk, salt, serum. These methods are: titration, rapid testing kits, electrochemical detection, flow injection analysis, inductively coupled plasma mass spectrometry (ICP-MS), capillary electrophoresis and radiochemical neutron activation analysis (Khazan et al., 2013). So, iodine content of salt can be determined by using both qualitative and quantitative method. Some trained or experience laboratory analysts are required to perform these methods in industry (Ajmal et al., 2014).

2.18 The Titrimetric Method

At first DeMaeyer described the titration method in 1979. According to many experts that titration method is one of the mostly used method to determine the quantity of iodine in iodized salt. Though it takes huge time to perform the whole procedures, this is easy to operate and comparatively it needs low cost (Khazan et al., 2013). First of all, free iodine is released from the salt and this free iodine is being titrated with sodium thiosulphate by using starch as an indicator. Finally, the result is expressed as parts of iodine per million parts of salt (ppm) or milligram iodine per kilogram salt(mg/kg). According Pieter et al., the titration method is surely the most popular method which is used for to determine the iodine concentration quantitatively in iodized salt due to its accuracy, relative ease of operation (Phillips, 2010). Almost every country around the world follow titrimetric procedures to determine iodine in iodized salt because this method has been globally established and become popular as the reference method. On the other hand, Rapid test kit is used in maximum household to determine presence of iodine in iodized salt (Haldimann et al., 2005).

2.19 Spectrophotometric Method

This is one of the best quantitative methods to determine the percentage of iodine in iodized salt (Jooste and Strydom, 2010). This method is also popular because it can determine the amount of iodine in the form of iodide or iodate. Different methods have been developed which are mainly based on the liberation of iodine from potassium iodide in any acidic medium (Jooste and Strydom, 2010). Koh et al., reported a spectrophotometric method was established based on the extraction oxidized iodine into carbon tetrachloride (CCl_4) and this proposed method was successfully applied in natural water sample to determine iodine (Koh et al., 1988). Another kinetic spectrophotometric method was developed based on catalytic outcome of iodide on the reaction with bromate in acidic medium. This method was proposed to determine the iodide percentage in food sample and their range would be 0.0 0.5-190 µg/L (Gurkan et al., 2004).

Different studies are available on the basis of iodine because iodine is important for the proper metabolism of human body. A study reported in 2009 to determine iodine content in retail salt samples in New Zealand and (ICP-MS) was used to determine the iodine label by taking 20 different salt samples. The average concentration of iodine in these salts were 32-36 mg/kg (Zimmenmann, 2009). Another study was reported where

iodometric titration method was used to determine the concentration of iodine in different salt brands of Bangladesh and it showed that only one brand contain low amount of iodine (Fardousi, 2012). Another study was found where iodometric titration method was used to determine iodine content of a salt sample which was collected from 12 different manufacturer of South Africa. The result showed that only 30.9% salt sample contained 40-60 ppm iodine (Jooste, 2003). Recently, a spectrophotometric method was developed to determine iodine content on the basis of electrochemical oxidation of iodide to iodine at a platinum electrode followed by the extraction of ionic associates of iodine-iodide complexes with (CCl_4) (Drozd and Tishakova, 2011).

Both iodometric titration and spectrophotometric method were used for the determination of iodine in commercially available eight salt samples in North Eastern Ethiopia. It had been found in final result that only 23.08 % salts were properly iodized and 61.54 % salt salts were inadequately iodized; On the other hand, 15.38 % salts were over iodized (Bediye and Berihe, 2015).

Chapter 3: Materials and Methods

3.1 Sample Collection

Iodized salt samples were collected from the local market of Chattogram Metropolitan Area. In total, 5 salt samples were collected. These salt were denoted as Brand A, Brand C, Brand F, Brand N, Brand Q. Stability of Iodine of these iodized salts was determined at an interval of 7 days. After collecting; samples were kept in transparent air tight dry plastic pot. The transparent plastic pot was stored under room temperature (28ºC-30ºC) in the Department of Applied Chemistry and Chemical Technology lab under faculty of Food Science and Technology in Chattogram Veterinary and Animal Sciences University.

3.2 Study Period

The research work was conducted from June to December, 2019 at the laboratories under the faculty of Food Science and Technology in Chattogram Veterinary and Animal Sciences University.

3.3 Titrimetric Method of Analysis

3.3.1 Principle

The test is an iodometric test. Iodometric titration is the widely used and simplest method which helps to determine the amount of iodine present in iodized salt. The purpose of the experiment was to standardize Sodium Thiosulphate by using Potassium Iodate. This is 'indirect process' where liberated iodine was used to standardize the sodium Thiosulphate. The initial stage in the reaction involved oxidation of iodide to iodine using potassium iodate. Iodine is slightly soluble in water, when potassium iodide is mixed properly to solubilize iodine; tri-iodide anion (I3-) is formed (Khazan et al., 2013). Salts are mixed with potassium iodide and for hydrolysis purpose sulfuric acid is added to the solution, iodine becomes free. By treating the solution with starch indicator gives a bluish black color. Hence existence of iodine is detected**.**

On iodometric process the following reactions occurs –

1. $IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$

(From Salt) (From KI) (From H_2SO_4)

2. $2Na_2S_2O_3 + I_2 \rightarrow 2NaI + 2Na_2S_4O_6$

In the initial step of reaction, free iodine is released from salt

- Salt sample contains iodate from which free iodine is released by reacting with H_2SO_4.
- Free iodine is being solubilized by adding extra KI iodine in the sample solution. In normal condition it is quite impossible for KI to be soluble in pure water.

In the second step, sodium thiosulphate is used against free iodine to run the titration process.

- In this titration process sodium thiosulphate neutralizes free iodine present in the sample solution. Liberated free iodine is calculated from the proportional relationship between amount of free iodine and the amount of sodium thiosulphate which neutralizes the free iodine.

In this reaction process starch is used for indication purpose and it gives deep blue color indicating reaction between starch and free iodine. Turning of this deep blue color solution into a clear solution indicates sodium thiosulphate has neutralized all of the free iodine. The related chemical equations at various steps are as follow,

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

$$I_2 + I^- \rightarrow I_3^-$$

$$I_3^- + 2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 3I^-$$

The overall reaction is, $IO_3^- + 5I^- + 6S_2O_3^{2-} \rightarrow 3S_4O_6^{2-} + 6I^- + 3H_2O$

3.3.2 Preparation of 10% Potassium iodide solution

10 gm of potassium iodide was dissolved into water and volume was adjusted up to100 ml.

3.3.3 Preparation of 2N sulfuric acid (H_2SO_4)

5.6ml of concentrated sulfuric acid was added drop wise into 50 ml of chilled distilled water and then the final volume was adjusted up to 100ml mark.

3.3.4 Preparation of potassium iodate solution

- 5g of potassium iodate was dried at 120˚C in hot air oven for 1hr.

- Then the reagent was cooled in desiccator
- Then it was dissolved in 100ml volumetric flask
- 50ml of stock solution was taken out and it was diluted by adding distill water which was adjusted to 1000ml.

3.3.5 Preparation of saturated salt solution (for processing techniques):

To determine the amount of iodine content in salt during boiling; 50 ml of distilled water was taken in a conical flask and 10g salt was taken from each sample. Then, the solution was heated for 10minutes. After boiling, the solution was cooled down at room temperature. Then the titration method was followed for boiling condition which was also used during normal atmospheric condition.

3.3.6 Preparation of 1% starch solution

To prepare 1% starch solution, 1g fresh starch was dissolved to 100ml hot distilled water. Here conical flask was used as solution vessel. Continuous stirring helped to dissolve the starch into hot water perfectly. This starch solution was then cooled up to room temperature for use.

3.3.7 Preparation of potassium iodate solution

- 5g of potassium iodate was dried at 120˚C in hot air oven for 1hr.
- Then the reagent was cooled in desiccator
- Then it was dissolved in 100ml volumetric flask
- 50ml of stock solution was taken out and it was diluted by adding distill water which was adjusted to 1000ml.

3.3.8 Preparation of 0.005 N Sodium Thiosulphate ($2Na_2S_2O_3$) solution

$2S_2O_3^{2-} \rightarrow S_4O_6^{2-} + 2e$

So, 2 × 248 g 2 $Na_2S_2O_3$.5H2O ≡ 2000 ml 1N

Or, 248 g $Na_2S_2O_3$.5H2O ≡ 1000 ml 1N

Or, 1.240 g $Na_2S_2O_3$.5H2O ≡ 1000 ml 0.005N

1.240 g of sodium thiosulphate pentahydrate was dissolved in 1000ml hot distilled water. 50 ml of stock solution was taken out and it was diluted to 1000ml distilled water in a volumetric flask.

3.3.9 Standardization steps of (0.005 N) Sodium Thiosulphate ($2Na_2S_2O_3$) solution

Sodium thiosulfate solution (.005N) was placed in a burette.25ml of potassium iodate solution was taken in a conical flask.2ml of sulfuric acid .2N was added and it was shaken to mix properly. Then it was titrated with 5ml of potassium iodide (10% solution) and again it was shaken to mix. After that it was titrated with sodium thiosulfate until the color became pale yellow. Then 1ml of starch solution was added. The mixture was turned to deep purple due to free iodine. The titration process was continued until the purple color was completely disappeared. The volume of sodium thiosulfate was noted.

Then the normality of sodium thiosulfate was calculated by the following method:

$S1 \times V1 = S2 \times V2$

So, $S1 = (S2 \times V2)/V1$

Here,

S1= Normality of sodium thiosulfate

V1=volume of sodium thiosulfate

S2= Normality of potassium iodate

V2= volume of potassium iodate

3.3.9 Standardization of Sodium thiosulfate solution

Table 3.1: Standardization of Sodium thiosulfate solution

Observation No.	Initial Burette Reading (ml)	Final Burette Reading (ml)	Difference (ml)	Average (ml)
01.	60.00	50.00	10.00	10.16
02.	50.00	39.60	10.40	
03.	39.60	29.50	10.10	

The strength of sodium thiosulfate is calculated using the following formula

Volume of sodium thiosulfate V1 = 10.16

Strength of potassium iodate S2 = 0.01N

Volume of potassium iodate, V2 = 10 ml

$S1 \times V1 = S2 \times V2$

So, $S1 = (S2 \times V2)/V1$

$= (0.005 \times 10)/10.16 = 0.0049N = .005N$

3.3.10 Procedure for Titration of Sample

First of all ,10g of salt sample was accurately weighed. Then it was transferred into a 250 ml clean conical flask. Distilled water was added to dissolve the sample in that conical flask. 1ml of 2N H2SO4 solution was added. The solution was kept for few minutes in normal temperature and after that, 5ml of 10% KI solution was added. Then the solution was shaken to mix properly the conical flask was covered with watch glass and was kept into a dark place for about 10 minutes. As the iodine was produced in the solution the color of the solution turned into deep yellow/brown.

Then the burette was properly cleaned and it was filled with 0.005N sodium thiosulfate solution. Then the sample solution was titrated against 0.005N thiosulfate solution. Titrate solution until the color of sample solution was turned into pale yellow.1ml freshly prepared starch solution was added to the mixture which turned the sample solution to purple/ deep blue color. This color indicates the presence of free iodine in the sample solution. Then sodium thiosulfate (drop by drop) was added very carefully. Thus, the titration process was continued until the solution became colorless. The whole process was repeated twice more and an average value for the volume of sodium thiosulfate solution was determined.

3.3.11 Calculation

From the average volume of $Na_2S_2O_3$ determined, the number of ppm of iodine in the salt sample was calculated with the following formula:

Iodine ppm = (R x100x1000x0.127xN)/6

Where, R = Average volume of $Na_2S_2O_3$

100 is used to convert the reading for 1000g of salt

1000 is used to convert gram of iodine to milligram of iodine

0.127 is used the weight of iodine equivalent to 1ml of normal Thiosulphate solution

N is the normality of Sodium Thiosulphate solution (which is 0.005N)

6 is used to arrive at the value that corresponds to 1 atom of iodine liberated

3.4. UV-Spectroscopic Method

In this method the amount of iodine is determined by using Uv visible spectrophotometer. Basically KIO_3, KI, boric acid (H_3BO_3), sulfuric acid (H_2SO_4), sodium thiosulfate ($Na_2S_2O_3$) and starch were used**.** These commercial salts were examined during the development of analytical method. Clean apparatus was used for this method. Deionized distilled water were used for the preparation of solutions. The measurements were carried out by using UV Spectrophotometer (SHIMADZU, Kyoto, Japan) with a 1 cm light path quartz cuvette at room temperature. The absorbance was measured at 352 nm for all the solutions.

The following solutions were prepared and used in the spectrophotometric procedures

3.4.1 Solution 1: Buffered KI solution

- 6.3 g of boric acid was dissolved in 700 mL distilled water.
- It was gently heated for boric dissolution and cooled to room temperature.
- 10 g of KI was added to this solution and after dilution it was made to 1 L with distilled water.

3.4.2 Solution 2: 0.1 M H_2SO_4

- 5.6 mL concentrated H2SO4 was added carefully to 900 mL distilled water.
- Then it was Completed to 1000 mL with distilled water.

3.4.3 Solution 3: 4.7 10-3 M KIO_3

- 0.500 g de potassium iodate (KIO_3) was added in 400 mL distilled water.
- Then it was Completed to 500 mL with distilled water.

3.4.4 Solution 4: 4.7 10-5 M KIO_3.

- Take 5.0 mL of Solution 3 solution was taken.

- After that 0.5 g KI was added.
- 10 mL of 0.1 M sulfuric acid (Solution2) was taken.
- Finally, it was dissolved and made to 500 mL with distilled water.
- it was prepared just before the calibration solutions.

3.4.5 Procedure 1 (preparation of Calibration solutions)

- First of all, some conical flask was taken and cleaned properly. After cleaning, those were dried.
- 5, 10, 15, and 20 mL of Solution 4 were taken into volumetric flask.
- Then it was completed to 100 mL with buffered solutions (Solution 1) in volumetric flasks.
- After that the labeling was done with permanent marker.

3.5.6 Procedure 2 (Preparation Blank Solution)

- 10 mL of 0.1 M sulfuric acid (Solution 2) was taken and it was made to 100 mL with distilled water in volumetric flask.
- Then the labelling was done with permanent marker.

3.5.6 Procedure 3 (preparation of Sample solutions)

- 0.25 g of iodate salt was taken in a beaker.
- 10 mL of 0.1 M sulfuric acid (Solution 2) was added.
- Then 50 mL buffered iodate solution (Solution1) was added.
- Finally, full mixture was transferred to a 100 mL volumetric flask and it was made to 100 mL with buffered solution.

Chapter 5: Results

5.1 Analysis of Different Salts

An elaborate and constructive analysis was made which represents required information about selected 5 branded iodized salts. The following figures illustrated that the concentration of iodine among different salts of Bangladesh and show their stability in different condition.

5.1.1 Brand A

Figure 5.1 represented the concentration of iodine determined in normal atmospheric condition which was 42.01 ppm after opening the packet of brand A and it was respectively reduced to 38.45 ppm ,31.76 ppm and 23.98ppm after every 1week interval.

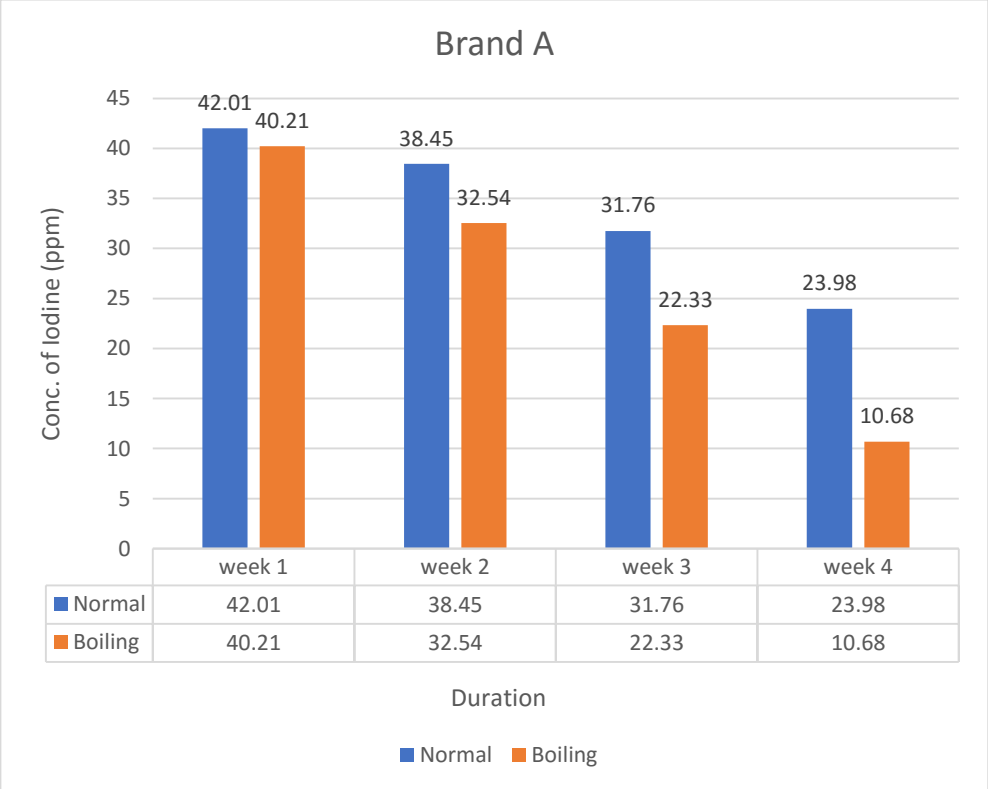

Figure 5.1: Stability of Iodine Condition in Brand A

Study results in Figure 5.1 also show that there was a significant loss of iodine during boiling. In this case, the value was also decreased gradually from 40.21 to 10.68 within four weeks. In this analysis, highest amount of loss is found during week 2 and week 3.

5.1.2 Brand F

The amount of iodine concentration in normal atmospheric condition was 44.45 ppm at first week and it was gradually decreased to 41.27, 38.47 and 19.06 ppm in 2nd, 3rd and 4th week.

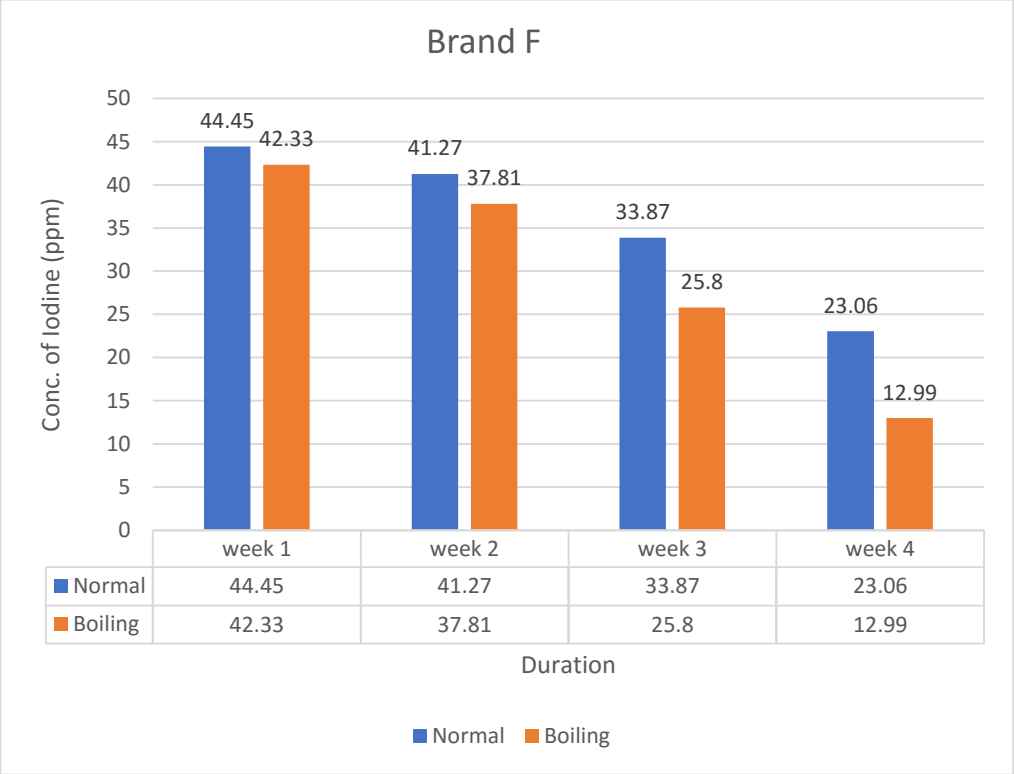

Figure 5.2: Stability of Iodine Condition in Brand F

Diagram 5.2 shows the content of iodine was higher after opening of the packet and a great loss of iodine had been noticed during last week of boiling. The highest value was found 42.33ppm at first week and 12.99 ppm iodine was recorded at the end of the last week. 37.81 ppm and 25.8 ppm were observed at 2nd and 3rd week.

5.1.3 Brand C

Figure-3 illustrates the changes of iodine loss in case of both normal and boiling condition. The concentration of iodine in normal atmospheric condition was 26.46 ppm at first week and a continuous reduction of loss was observed in every and the value was 22.111ppm, 16.93 ppm and 8.35 ppm

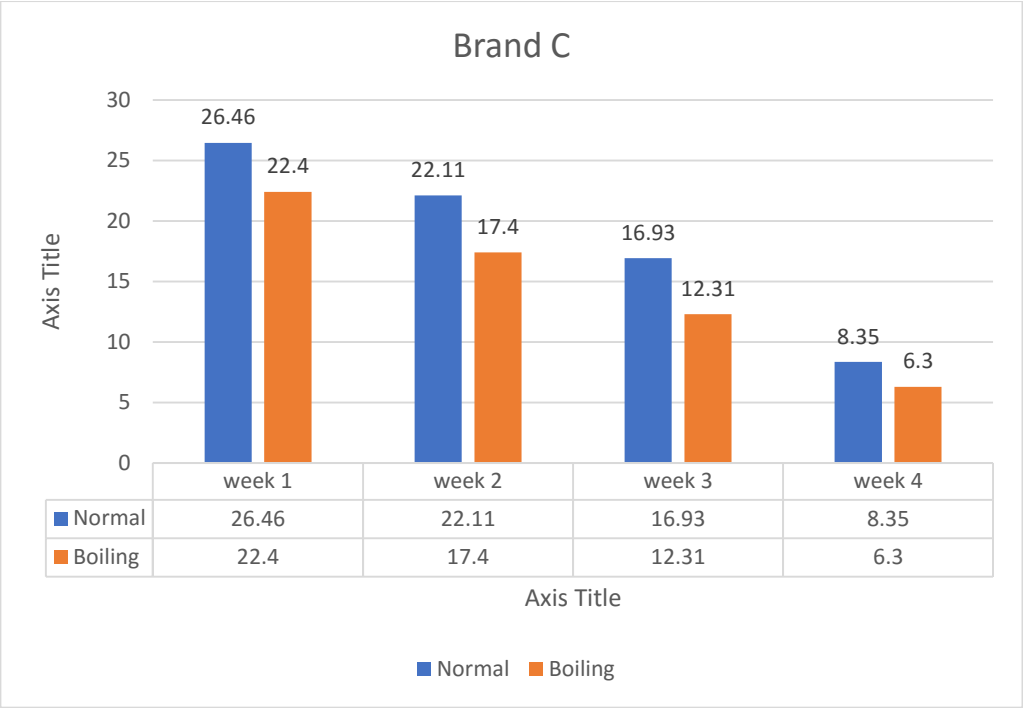

Figure 5.3: Stability of iodine condition in Brand C

This diagram represents that the result was sharply decreased to 6.3 ppm in case of boiling at the end of 4th week where the initial value was 22.4ppm after opening the packet. The same kind of changes occurred in second and third week.

5.1.4 Brand N

Iodine content of brand N is expressed in figure-4. Highest content of iodine was 24.34 ppm after opening of the packet and lowest concentration was noticed during last week which was 6.31ppm.

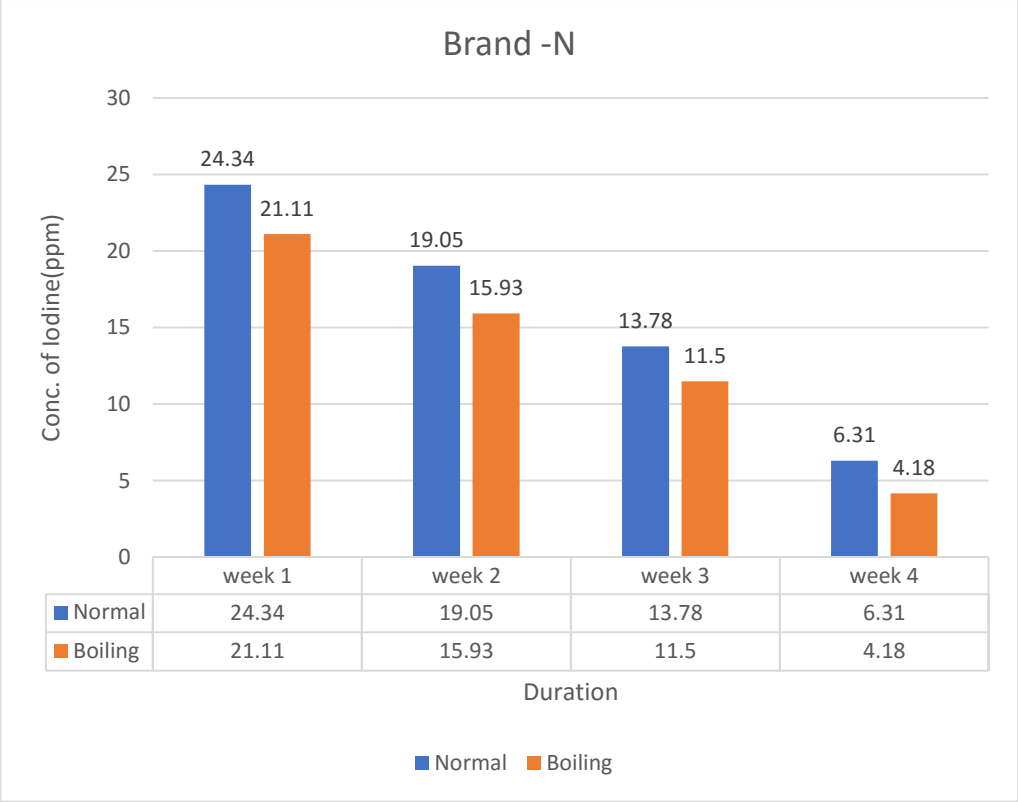

Figure 5.4: Stability of Iodine Condition in Brand N

In case boiling, the value was also highest at first day which was 22.21 and gradually it was decreased to 15.93 ppm,11.5 ppm and 4.8 ppm.

5.1.5 Brand Q

Iodine content of Brand Q in normal atmospheric condition and boiling condition was different from each other, higher iodine content was 43.39 ppm at first week and it was changed to 16.93 ppm in last week during normal environment.

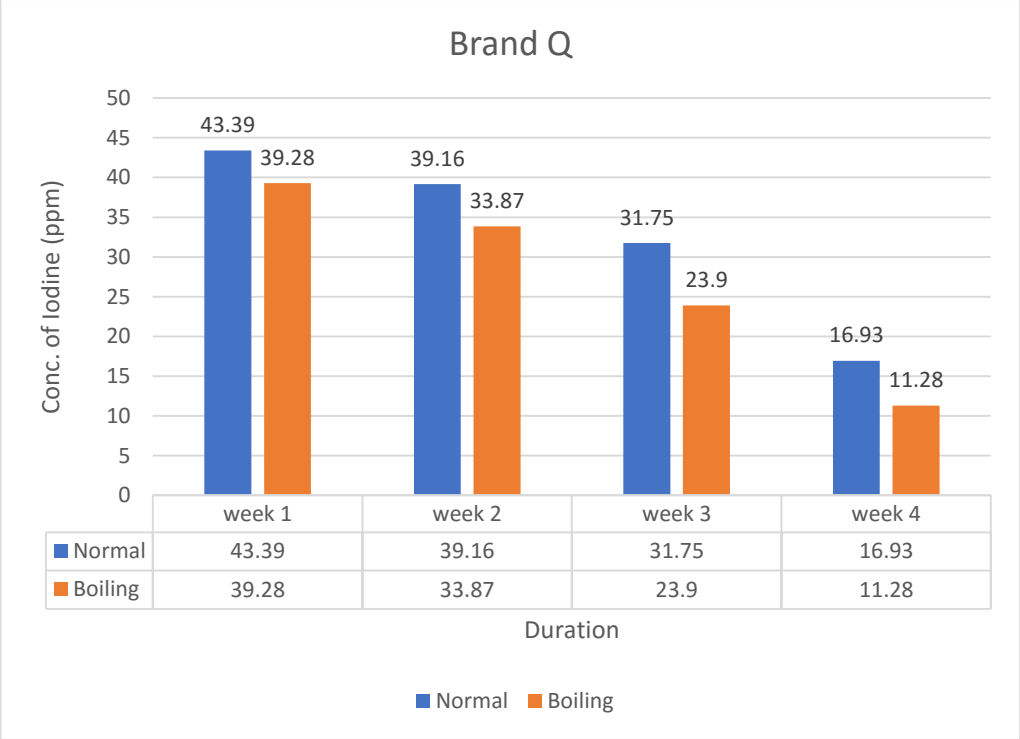

Figure 5.5: Stability of Iodine Condition in Brand Q

The value was also declined from 41.28 to 14.82 from 1st week to 4th week. A lower content of iodine was examined after third week during boiling methods which was 11.28 ppm though after opening the value was 39.28 ppm.

5.2 Percentage of Iodine Loss

This following diagram reveal the percent loss of iodine in salt samples which were analyzed and critically examined in well set-up laboratory. It is clear that majority of the salt samples showed required iodine availability compared with their indication level (20-50 ppm).

5.3 Percentage of iodine stability reduction in normal atmospheric

The present study deals with measurement of iodine availability and iodine stability of these iodized salts at different conditions. The following table represents percent loss of iodine stability of iodized salts during normal atmospheric condition at specific time interval (1 week).

Table-5.1: Comparison Table for Percent Reduction Rate of Iodine of Iodized Salts at Different Time Period in Normal Atmospheric Condition.

Iodized salt Samples	Percent of iodine stability reduction during normal atmospheric Condition			
	After opening the packet (Week 1)	Week 2	Week 3	Week 4
Brand A	0%	8.47%	24.70%	42.92%
Brand C	0%	16.44%	36.02%	68.4%
Brand F	0%	7.15%	23.80%	48.12%
Brand N	0%	18.04%	43.38%	74.08%
Brand Q	0%	9.75%	26.83%	60.9%

In this analytical study, reduction percent of iodine was 0% in Brand A and respectively increased to 8.47%%, 24.40%, 42.92% whereas in case of Brand F the percentage was 0% at first day and after 1-week interval it became 7.15%, 23.80% and 48.12%% respectively during normal atmospheric condition. Higher stability was found in case of Brand F among all samples.

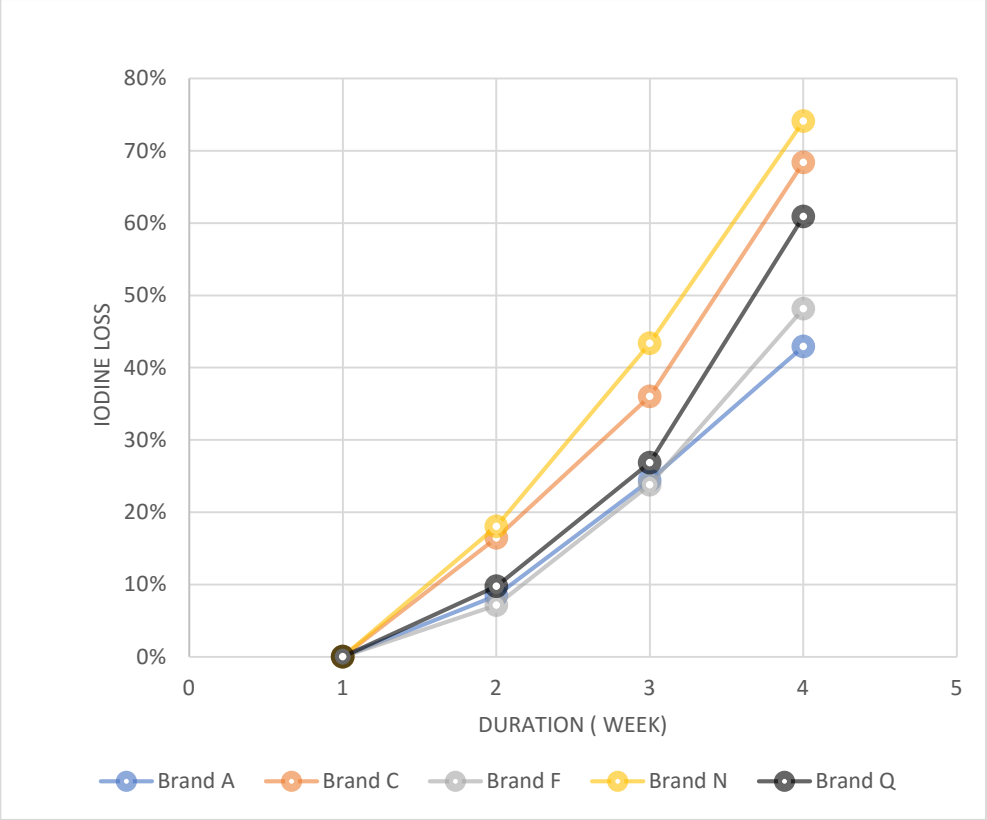

Figure 5.6: Graphical presentation of Iodine Reduction in Normal Atmospheric Condition up to 1-week Interval

In this experimental study, the result showed that iodine was less stable in Brand N during different storing time period. After keeping it in normal atmospheric environmental condition percent loss of iodine stability was 18.04%, 43.38% and 74.08%.

It was also noticed that iodine was less stable during different storing time period. After keeping in normal environment for 1, 2, 3 and 4-weeks iodine stability was reduced consequently and the average reduction percentage was 42.92%, 68.4%, 48.12%, 60.9% and 74.08% for Brand A, Brand C, Brand F, Brand Q and Brand N Fine iodized salts respectively.

After keeping the salt samples in normal atmospheric environment for first and second weeks. Brand Q salt showed greater iodine stability and Brand C was less stable. After third and fourth week both Brand Q and Brand F showed the greater iodine stability whereas Brand N and Brand C became less stable.

5.4 Percentage of Iodine Stability Reduction During Boiling

In the present study, the loss of iodine during cooking was assessed. The minimum loss of iodine found for Brand F and Brand Q was 12.95 % and 13.77 % after 1st week while maximum loss of iodine was observed 80.20% during boiling in Brand N after third week.

Table-5.2: Comparison Table for Percent Reduction Rate of Iodine of Iodized Salts at Different Time Period During Boiling Condition.

Iodized salt Samples	Percent of iodine stability reduction during normal atmospheric Condition			
	After opening the packet (Week 1)	Week 2	Week 3	Week 4
Brand A	0%	19.07%	44.47%	73.45%
Brand C	0%	22.32%	45.04%	71.88%
Brand F	0%	12.95%	31.76%	69.31%
Brand N	0%	24.54%	45.52%	80.20%
Brand Q	0%	13.77%	39.15%	71.28%

After opening, it was found that the reduction percent of iodine was 0% in Brand A and respectively increased to 19.07%, 44.47%, 73.45% whereas in case of Brand F the percentage was 0% at first day and after 1-week interval it became 12.95%, 31.76 % and 69.31 % respectively during boiling condition (Table-5.2).

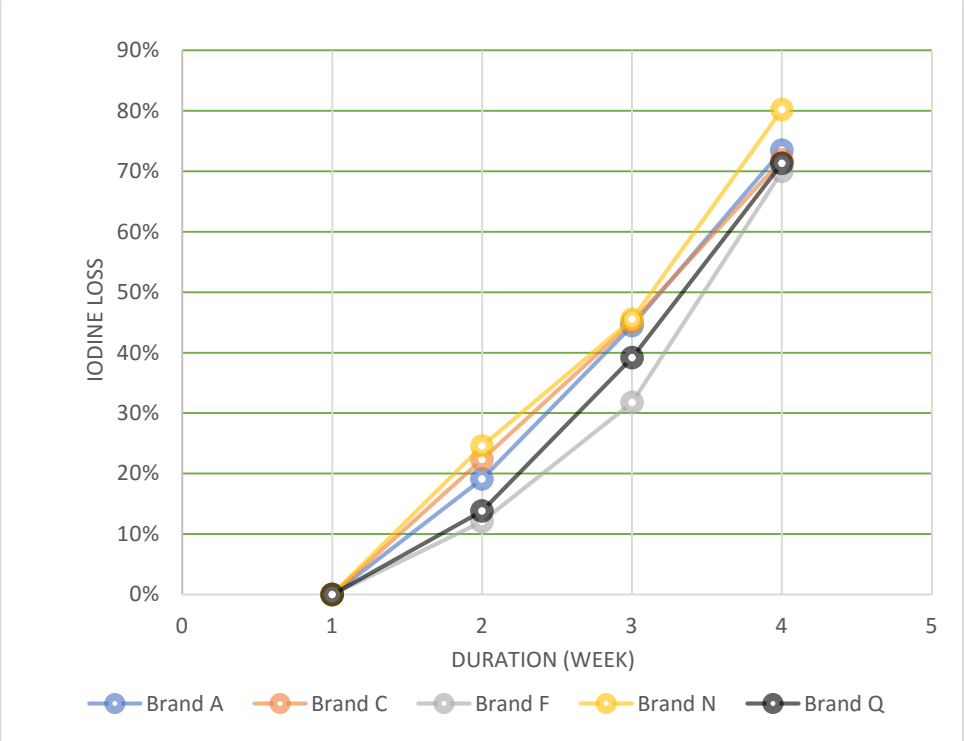

Figure 5.7: Graphical presentation of iodine stability reduction during Boiling up to 1-week time interval

The analysis revealed that the loss of iodine was ranged from 22.32% to 71.88% for Brand C from 1st to 3rd week. It also expresses that Brand A was more stable in first 2 weeks where the percent loss was 19.07 % and 44.47 % but the loss of iodine increased highly after 3rd week which was 73.45%.

Figure 5.7 illustrates that iodine stability was reduced consequently and the average reduction percentage was 73.45%, 69.31%, 71.88% ,71.28% and 80.20% for Brand A, Brand C, Brand F, Brand Q and Brand N Fine iodized salts respectively during boiling condition.

5.5 Comparison of iodine concentration (using both UV Spectrophotometric and Titrimetric method)

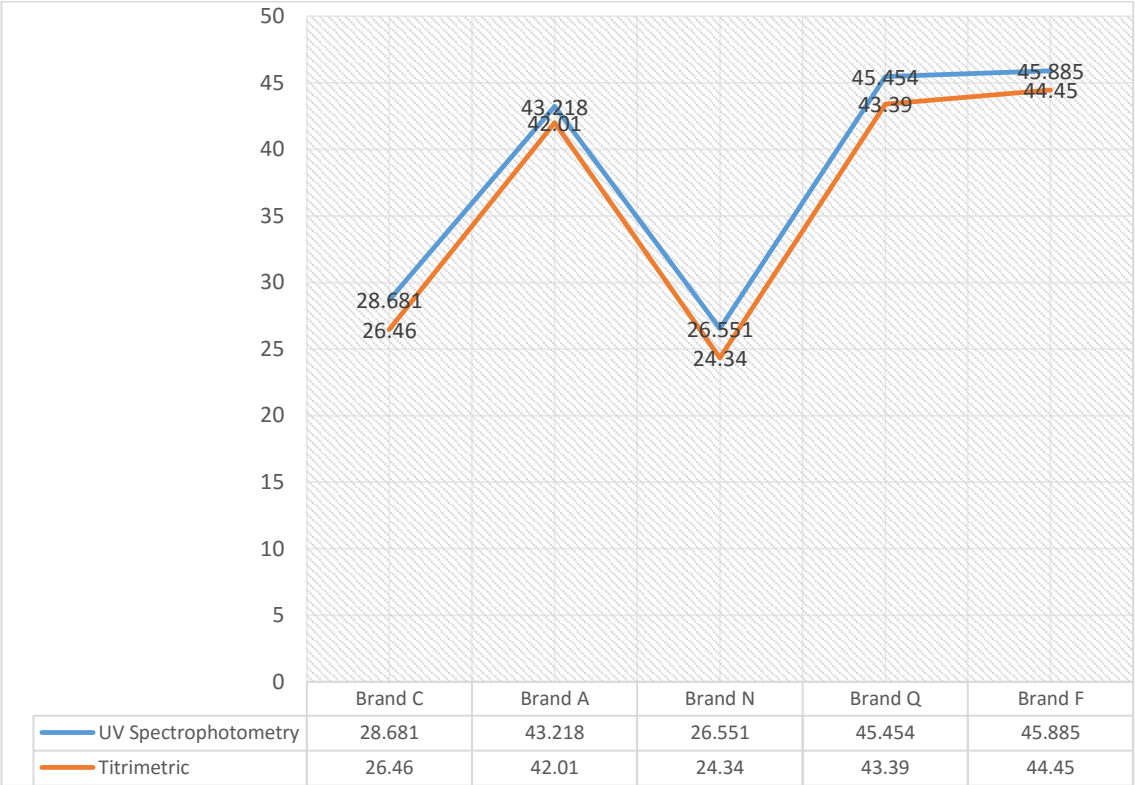

Figure 5.8: Concentration of Iodine in Normal Atmospheric Condition by Using Both Titrimetric and UV-Spectrophotometric Method

UV spectrophotometric method was used to compare the concentration of iodine value with titrimetric method. In normal atmospheric condition the amount of iodine for Brand C was 28.681 ppm when the value of iodine was found 26.46 ppm in case of Trimetric method. The figure-5.8 shows the iodine content of Brand A which was observed 43.218 ppm and the amount was determined 26.651ppm for Brand N in case of UV spectrophotometric method. In sample Q amount iodine was 45.454ppm and the concentration in Fresh salt was determined 45.885 ppm. The figure illustrated that the concentration of iodine in UV spectrophotometric was slightly higher than titrimetric method.

Chapter 5: Discussion

Iodized salt is the major source of iodine especially in developing country like Bangladesh. Now-a-days it has become an alarming issue that a lot of people are suffering from IDDs. The Government of Bangladesh is officially committed to eliminate IDDs with the collaboration of national and international organization. The current study was conducted to investigate the concentration of iodine in different branded salts of Bangladesh. This study also examined the stability of iodine in salt during specific time interval. The concentration of iodine was differed in every processing technique. Finally, a comparison was established between two methods in iodine concentration among these salts.

In 1989, the Government of Bangladesh passed a law making it mandatory that all edible salt must be iodized. The law stipulates that all salt for human consumption must contain (45-50) parts per million (ppm) of iodine at the time of production and not less than 20 ppm iodine at the time of retail, to ensure a minimum of 15 ppm iodine at the household level (National, 2011).

In this study it was found (fig-2) that the concentration of iodine was higher (44.45 ppm) for Brand F salt than other salts in normal atmospheric condition. This result is in agreement with study conducted by Bashar et al. (2016) and Jabin et al. (2009). Out of the 5 samples, Brand N showed comparatively poor content of iodine (24.34 ppm) concentration from other samples.

The result of this study indicates that in Brand A salt samples, the concentration of iodine was 42.01ppm in first week at normal atmospheric condition. However, this reduction might be occurred due to the presence of hygroscopic impurities like magnesium chloride ($MgCl_2$) reducing agents like ferrous ions or lower pH that enhanced the reduction of iodate. Factors like impurities, reducing agents, metal ions and pH value vary from one salt product to another (Diosady et al., 1998).

The iodine content of Brand C was comparatively higher (26.46 ppm) than Brand N (24.34ppm). On the other hand, iodine content of Brand Q was much higher (43.39) than both Brand N and Brand C. This study result corroborates the observations of the study conducted by Fardousi (2011). It is important to monitor whether the iodized salt produced and sold in the market contains the specific quantities of iodine as per law i.e.

45- 50 ppm at the time of production level, 20 ppm at the time of retail shop level and a minimum of 15 ppm at the time of consumption level (Jabin , 2009). In first week, the amount of iodine was higher for every sample but gradually the concentration of iodine decreases. Concentration of iodine was measured by using both titrimetric and spectrophotometric methods in normal atmospheric condition. A little difference in iodine concentration is observed between these methods. The amount of iodine content is shown slightly higher in UV spectrophotometric method than titrimetric method for all samples. Bashar et al. reported that a very good agreement is observed between the spectrophotometric method and titrimetric method.

The Bangladesh Standard Testing Institute-BSTI standard for iodine rich salt is 20 to 50 ppm. In result it is shown that iodine content of salt samples is degraded with respect to storage period. Both Graph-5 represents percent loss of iodine stability of iodized salts during normal atmospheric condition at specific time interval (1 week) where the gradual increase of percent loss of iodine was noticed for every sample. At the end of last week, the maximum loss percentage (74.08%) was recorded for Brand N. It was observed that iodine content in a new and freshly opened pack of salt is more than a pack which was opened 1 or 2 weeks before (Yasif et al., 2016).

It was found that iodine was less stable in Brand N during different storing time period. After keeping it in normal atmospheric environment for 1, 2, 3-week, percent loss of iodine stability was 18.04%, 43.38% and 74.08% respectively. Jabin et al. (2009) reported that iodine content of open salt is zero and iodine content of iodized salt reduce gradually day by day due to moisture and impurities present on it reported by Prodhan et al. (2014) found 54 ppm iodine in ACI iodized salt that is nearer to the present study. Slight variation might be due to improper packaging or personal error in determination of iodine content (Bashar et al., 2016).

From figure 5.8 it can be summarized that the concentration of iodine measured in Brand A, Brand F, Brand C, Brand Q and Brand N was 42.01ppm, 44.45ppm, 26.46ppm, 44.39 ppm and 24.34 ppm after opening the packet when iodometric method was used. In case of UV spectrophotometry, the concentration of iodine was observed 43.218 ppm, 45.885 ppm, 45.454 ppm, 26.68 ppm and 26.551 ppm among those similar samples. Yasif et al. (2016) reported in his study that the minimum loss of iodine was found during shallow frying (2.20% to 7.33%) while maximum loss of iodine was

found during boiling (14.33% to 32.58%). This could be due to the fact that during boiling, water was used for cooking the food. Salt is hygroscopic in nature and soluble in water. Hence, it absorbed water and the iodine present in the salt was leached out and lost during boiling. But water was not required as a cooking medium during shallow frying, so loss of iodine during boiling was more than during shallow frying (Yasif et al., 2016).

During Boiling condition, the concentration of iodine in these iodized salts was significantly reduced than in normal atmospheric condition. Significant loss of iodine is shown in Brand N and confidence salt in 3rd to 4th week. The minimum loss of iodine found for Brand F and Brand Q was 12.95 % and 13.77 % after 1st week while maximum loss of iodine was observed 80.20% during boiling in Brand N after third week. This study also revealed that the maximum concentration of iodine is observed in Fresh salt during boiling condition and the concentration of iodine is minimum in Brand 1. However, this finding is lower than the iodine concentration of previously reported by Diosady et al. (1998).

From this measurement it was observed that in boiling by same procedure, loss of iodine varied from a small amount to a large amount. The iodine content of all samples was decreased due to boiling. The percentage of iodine loss increases when the temperature rises. The highest percent loss of iodine was 71.88 % observed in Brand C salt after 3rd week though the loss of iodine was recorded 22.32 % during first week. The figures 6 also indicated that iodine loss was maximum during boiling as iodine was exposed to high temperature. The minimum loss of iodine was noticed for sample A during normal condition which was 42.92 % but when heat was applied the stability was highly declined and the percent loss of iodine became 73.45%. Yasif et al (2016) narrated that loss of iodine was minimum in case of shallow frying due to small amount of heat and less time of exposure and he also revealed that besides degree of heat applied, loss of iodine also varies in various recipes. This was occurred due to a possible binding of iodine with the various components of food. Moreover, food contains many other ingredients especially many unsaturated compounds. These instaurations may also form complexes with iodine rendering less evaporation. Further research is needed to postulate this possibility.

The percent loss of iodine concentration reduced in 11.9 % at 70˚C, 17.7 % at 80 ˚C, 21.7 % at 90 ˚C conducted by Prodhan et al (2014). In this present study, it can be expressed from figure (5) and (6) that on 1st day iodine was slightly reduced in boiling condition than in normal condition. But from 2nd to 4th week the concentration of iodine was gradually reduced in every sample during cooking. So, the concentration of iodine in different branded salts were followed the order Brand F> Brand Q > Brand A> Brand C >Brand N.

Chapter 6: Conclusion

Iodine is an indispensable microelement to human which is required tiny in amount for the betterment of individuals to prevent IDDs. Iodization of salt is most important population-based intervention to eliminate IDD and it has been shown as an effective tool for reducing IDDs.

From the experimental data it can be concluded that, the presence of iodine has been confirmed for all of the samples which were collected from local market of Chittagong. The present study brings out successful application for both UV-Vis Spectrophotometric method and Iodometric titration method for the determination of iodine in each salt sample which unravel amount of iodine content. The study elucidates stability of iodine was reduced during boiling and cooking condition. The amount of iodine is greater in normal atmospheric condition rather than boiling and cooking condition. Iodine stability is gradually reduced when the sample was kept for long time. In this study it was found that the impacts of storage condition directly associate with iodine loss of the salts. Here all salt samples were good in iodine content but among them Fresh salt contains more iodine compare to others in every processing technique. Loss of iodine is lower in ACI salts, Queen salts and Fresh salts compare to others of same category. By maintaining the optimum moisture content, minimum impurities level and proper storage condition iodine loss of salt can be minimized.

Chapter 7: Recommendations and Future perspectives

According to the study results; the following recommendation have been suggested:

- Further studies should be carried out in order to investigate factors affecting iodine concentration in salt.
- Mass awareness and social consciousness are the most effective way to avoid iodine reduction during different cooking and storage condition. It is not solely the responsibility of the government but media, public representatives and societies must also be responsive to avoid consuming non-iodized open salt.
- The concerned authority should establish approaches to ensure appropriate iodine fortification and regular market monitoring.
- Sprinkle salt on food after cooking (wherever possible) rather than adding salt while cooking to prevent iodine losses.
- Further, storage of iodized salt in a hot and humid condition near the cooking oven should be avoided.

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Appendices

Appendix A : Experimental data on concentration of Iodine during normal atmospheric concentration

Code of used iodized salt	Claimed amount of iodine content(ppm)	Amount of iodine content (ppm) just after opening the salt packet	Amount of iodine content (ppm) one week later	Amount of iodine content (ppm) two weeks later	Amount of iodine content (ppm) two weeks later
Brand A	(20-50)	42.01	38.45	31.76	23.98
Brand C	(20-50)	26.46	22.11	16.93	8.35
Brand F	(20-50)	44.45	41.27	33.87	23.06
Brand N	(20-50)	24.34	19.95	13.78	6.31
Brand Q	(30-50)	43.39	39.16	31.75	16.93

Appendix B : Experimental data on concentration of Iodine during Boiling

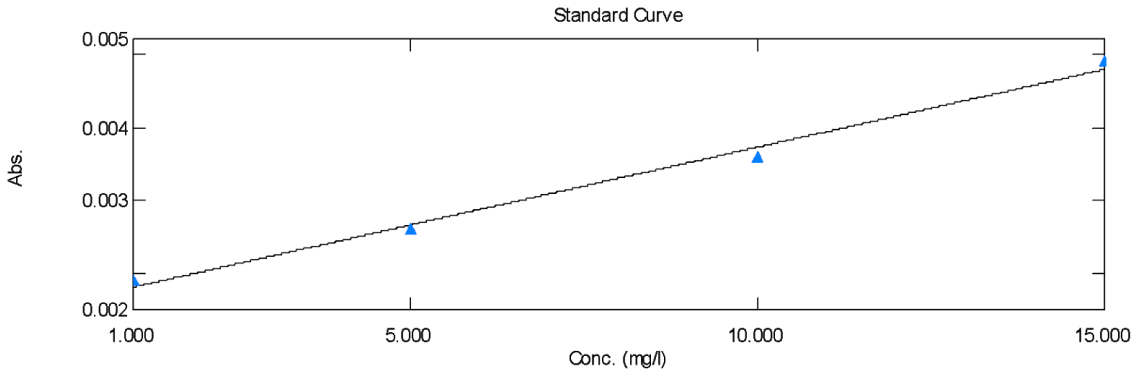
Code of used iodized salt	Claimed amount of iodine content(ppm)	Amount of iodine content (ppm) just after opening the salt packet	Amount of iodine content (ppm) one week later	Amount of iodine content (ppm) two weeks later	Amount of iodine content (ppm) two weeks later
Brand A	(20-50)	40.21	32.54	22.33	10.68
Brand C	(20-50)	22.40	17.40	12.31	6.30
Brand F	(20-50)	42.33	37.81	25.8	12.99
Brand N	(20-50)	21.11	15.93	11.50	4.18
Brand Q	(30-50)	38.28	33.87	23.9	11.28

Appendix C : Iodine Conc. In Different Salt Samples

Table of Standard:

Observation No.	Sample ID	Type	Conc.	WL352.0	Wgt.Factor
1	S1	Standard	1.000	0.002	1.000
2	S2	Standard	5.000	0.003	1.000
3	S3	Standard	10.000	0.004	1.000
4	S4	Standard	15.000	0.005	1.000

Standard Curve:



y = 2.12641e-004 x + 0.00160224
r2 = 0.99108

Table of Sample:

Observation No	Sample ID	Type	Conc.	WL352.0
1	Brand C	Unknown	28.681	0.008
2	Brand A	Unknown	43.218	0.011
3	Brand Q	Unknown	45.454	0.011
4	Brand N	Unknown	26.551	0.007
5	Brand F	Unknown	45.885	0.011

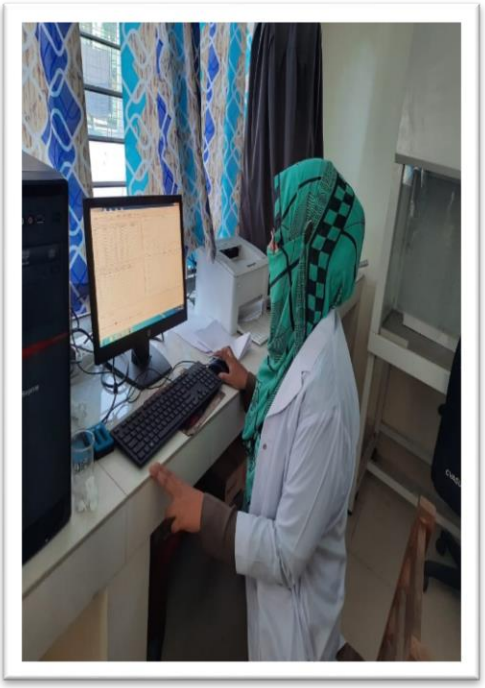
Appendix D: Sample Collection

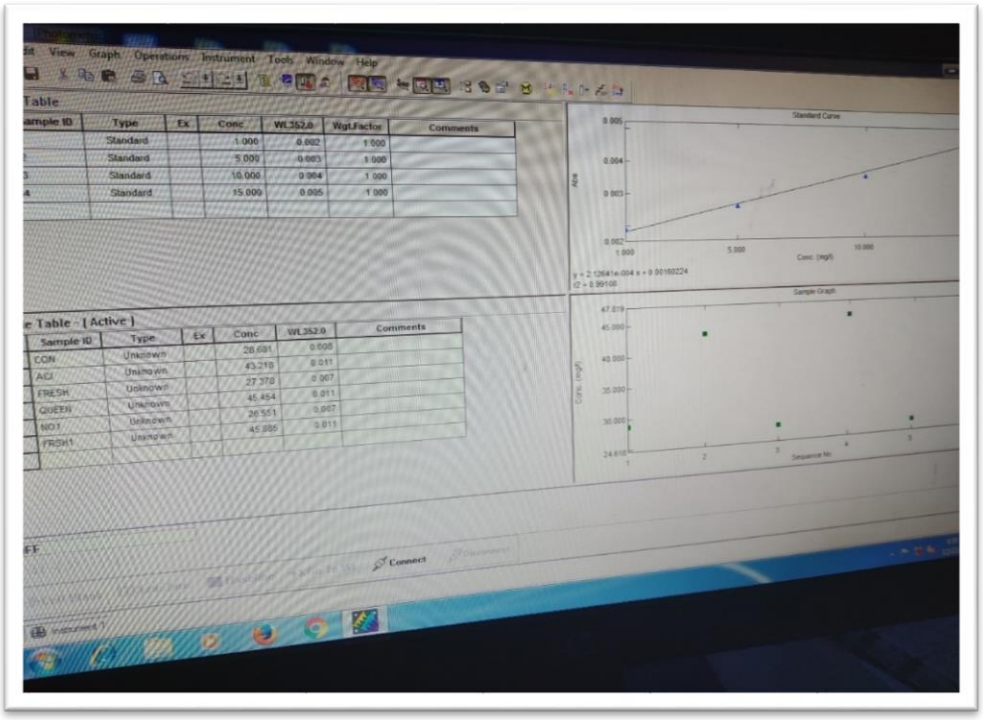
Appendix E : Sample Preperation for Titrimetric Method

Appendix F: Steps for Titrimetric Method

Appendix G: Steps for UV – Spectrophotometry method

Appendix H: Sample analysis by using UV- spectrophotometer





Brief Biography

Sharmin Yesmin Shanta completed B.Sc. (Hons.) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh with CGPA 3.73 out of 4.00. Now, she is a candidate for the degree of MS in Applied Human Nutrition and Dietetics under Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has immense interest to work in public health perspective like to investigate factors affecting iodine concentration in salt, Iodine fortification in oil, and also in the area of product development, natural preservatives, nutritional value analysis, relation of food properties and processing, quality control and quality assurance regarding food, taste and flavour.