ADDIS ABABA UNIVERSITY

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Determination of Iodine Content in Salt Samples Commercially Available in Debre Tabor Town, South Gondar, Ethiopia

By: Amanu Monie, B.Sc.

A Thesis Submitted to Addis Ababa University, School of Graduate Studies, in Partial Fulfillment of Requirements for Degree of Master of Science in Medical Biochemistry

March, 2020

Addis Ababa, Ethiopia

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Declaration Sheet

This is to certify that this thesis entitled "**Determination of iodine content in salt samples commercially available in Debre Tabor town, South Gondar, Ethiopia"** was conducted by Amanu Monie Wassihun, and Submitted to department of biochemistry in partial fulfillment of the requirements for the degree "Master of Science in Medical Biochemistry" complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Examiner: Dr. Hagos Tesfay Signature_____ Date_____

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LIST OF ABBREVIATIONS AND ACRONYMS

DIT----------Di-iodotyrosine

EFMH----------Ethiopian Federal Ministry of Health

ICCIDD----------International Council for Control of Iodine Deficiency Disorder

IDD----------Iodine Deficiency Disorder

MIT---------- Mono-iodotyrosine

ppm ----------Parts per million

QSAE----------Quality and Standard Authority of Ethiopia

RTK----------Rapid test kit

T_4----------Tetra-iodothyronine or thyroxine

TSH----------Thyroid Stimulating Hormone

T_3----------Tri-iodothyronine

UNICEF----------United Nation International Children's Emergency Fund

USI----------Universal Salt Iodization

WHO----------World Health Organization

ABSTRACT

Background: Iodine is essential trace element important for synthesis of thyroid hormones. Human body cannot synthesize iodine; it is obtained from different sources. Inadequate consumption of iodine leads to iodine deficiency disorder. Iodine deficiency disorder is major global public health problem and it is possible to root out this problem by universal salt iodization.

Objective: The main aim of this study is to determine iodine content in salt samples commercially available in Debre Tabor town using rapid test kit (RTK) and iodometric titration.

Methodology: The study was conducted in Debre Tabor town from December 16 to 29, 2019 and cross- sectional study was used to determine the iodine content in salt samples purchased from different shops of Debre Tabor town. All statistical analyses were analyzed using the software, Statistical Package for the Social Science (SPSS) ^{version} 21 and results were expressed as mean ± standard deviation. Statistical difference between mean iodine content of salt was analyzed by One Way Analysis of Variance (ANOVA) and statistical significance was accepted at $p < 0.05$.

Result: Iodine content of different salt samples determined by iodometric titration was found between 11.435 ± 0.441 mg/kg to 37.252 ± 0.603 mg/kg. The highest value was recorded for Risa (37.252 ± 0.603 mg/kg) while the lowest value for Ediget salt (11.435 ± 0.441 mg/kg). Iodine content of salts determined by rapid test kit (RTK) showed that for almost all of salt samples iodine content was >15 mg/kg except for Ediget salt <15 mg/kg. Of ten salt samples (n =10) collected, according to WHO, (n =9), 90% were adequately iodized (15-40 mg/kg), and one salt sample (n =1), 10% was insufficiently iodized (5-14.9 mg/kg). According to iodization level set in Ethiopia (36-48 mg/kg) only one salt sample (Risa, 37.252 ± 0.603 mg/kg) was adequately iodized.

Conclusion: The iodine content of salts available in Debre Tabor town was determined using rapid test kit and iodometric titration. Different salt brands contained different amount of iodine and almost all of salt samples collected from study area did not contain adequate amount of iodine according to iodization level set in Ethiopia. But most of salt samples contained adequate level of iodine according to WHO's level of iodization.

Key words: - Iodine, Iodine deficiency disorder, Iodometric titration, Rapid test kit

1. INTRODUCTION

1.1. Background

Iodine is an essential trace element required in small amount. Healthy humans require iodine, as crucial component of thyroid hormone needed for production of tri- iodothyronine (T_3) and tetra-iodothyronine (T_4) and for thyroid gland proper functioning (Aweke *et al*., 2014). About 15-20 mg of iodine is found in total body of healthy person, 70-80% is present in the thyroid gland (Mohammed, 2016). But the human body cannot synthesize iodine and it cannot be stored for long periods; thus, it is necessary to obtain it from different sources. Dietary intake is the primary source of iodine. Sea foods, dairy products, and plants grown in iodine rich soil are common source of iodine (Duressa *et al*., 2014; Meselech Regassa *et al*., 2016; Mohammed, 2016). So, iodine rich foods are important to get recommended iodine. But the value of recommended intake of iodine for different group of population (infants, children, adults, pregnant and lactating women's) is different according to age and also for females during pregnancy and lactation (Fardousi, 2012).

Iodine is necessary for thyroid gland to produce thyroid hormone. Production of thyroid hormone can be decreased if there is inadequate consumption of iodine. This decreased production of thyroid hormone affects human metabolism, development and functioning of brain, heart, liver and kidney; which leads to iodine deficiency disorder (IDD) like goiter, cretinism, abortion, still birth, psychomotor defect and impaired mental function (Ahmad *et al.,* 2012; Abebe *et al*., 2017). It is reported that at least 350 million Africans are at risk of iodine deficiency (Agbozo *et al.*, 2017). According to national survey made by Ethiopian nutrition institute, one out of every 1000 is cretin, and about 50,000 perinatal deaths are occurring annually due to IDDs (Gerensea *et al*., 2016; Demissie, 2019)

Universal salt iodization (USI) is the most widely practical intervention and highly beneficial measure in eliminating IDDs (WHO, 2007; Bani, 2014; Maheswari *et al*., 2019). World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF) and International Council for Control of Iodine Deficiency Disorder (ICCIDD) recommended universal salt iodization as the safe and cost effective measure to eliminate IDD (Maheswari *et al.*, 2019). In order to prevent IDD, salt iodine testing is important process which is indicator for

monitoring progresses towards USI (Aweke *et al*., 2014). After making allowance for losses of iodine during storage and distribution, the quality and standard authority of Ethiopia has set iodine level to be between 60 - 80 ppm as potassium iodate or 36 - 48 mg/kg as iodine. The average consumption of salt per person per day is 10 grams and salt with iodine content of 60 mg/kg would satisfy the recommended daily requirement of 150µg of iodine per adult to prevent IDD (Gerensea *et al.*, 2016).

Consistent monitoring of iodine in salt at production, storage, sale, and consumption level is vital component of iodization program that should be adjusted to meet local condition and requirements. Therefore, monitoring salt iodization is useful and first step in tracking progress towards meeting goal of IDD elimination or prevention. Consumption of iodized salt is the best way to eliminate iodine deficiency disorders. Iodate and iodide are the two forms in which iodine is added to salt. The content of iodine added as iodate or iodide to the salt can be affected by heat, light, time and other environmental factors. One study conducted in Palestine showed that to determine the content of iodine out of 99 salt samples collected from Palestine consumers, it was found that 23-28% of samples have lost iodine content by 61-80% and 9% have lost up to 100%. And about 43.5% of samples fit to Palestinian standards institute recommendation (35-55ppm) while 58% fit UNICEF recommendation (15%) (Rajabi, 2016). Another study conducted in Bangladesh showed that Out of the 7 samples, only one has shown to have poor content of iodine, but that may also be due to long exposure times to moisture or heat while working in the laboratory (Fardousi, 2012). Salt iodization is common practice in Ethiopia and monitoring the level of iodization is critical thing in eliminating IDD using appropriate method of salt iodine content analysis. There are different salts from inland and foreign countries that are commercially available in Debre Tabor town for human consumption. In this study, the iodine content of the salt available in the study area was determined using rapid test kit (RTK) and iodometric titration. The estimation of the salt iodine by using RTK is based on the reaction between starch and iodine to form starch-iodine complex. RTK solution contains an acidic buffer, reducing agent, which convert potassium iodate to iodine, and starch. Iodometric titration is the most frequently used method to determine iodine content in salt because of its accuracy. This method is recommended at various levels of a distribution system (Khazan *et al*., 2013). Mostly it is required to monitor the adequacy of salt iodization at the level of production, importation and consumption (Rohner *et al*., 2015).

1.2. Literature Review

1.2.1. Iodine

Iodine is an essential trace element required in small amount. Healthy humans require iodine, crucial component of thyroid hormone needed for the production of T_3 and T_4, and for thyroid gland proper functioning (Aweke *et al.*, 2014). These hormones play role in the process of early growth and development of most organs (Gerensea *et al*., 2016). About 15 - 20 mg of iodine is found in total body of healthy person; 70 - 80% present in the thyroid gland (Mohammed, 2016).

Common sources of iodine are water and diets that are dependent on soil iodine levels. Iodine is widely but unevenly distributed in the earth's environment. Leaching, glaciations, flooding, and erosion in many regions deplete surface soils of iodine (Mohammed, 2016). The native iodine content of most foods is low, while foods from marine origin have higher content (Kapil, 2008, Pesce and Kopp, 2014). Despite this, about 90% dietary iodine is derived from foods and 10% from drinking water. The human body cannot synthesize iodine and it cannot be stored for long periods; thus, it is necessary to obtain it from different sources, with food being the most important and tiny amounts are needed regularly. Dietary intake is the primary source of iodine. Sea foods, dairy products and plants grown in iodine rich soils are common source of iodine (Duressa *et al.*, 2014; Meselech Regassa *et al*., 2016).

Table 1: Sources of iodine

Source of iodine	Amount of iodine
Kelp and other weeds	1-2 mg/kg
Shellfish	0.79-1.6 mg/kg
Sea salt	1.4 mg/kg
Egg	93 µg/kg
Poultry and meat	50 µg/kg
Grain and cereals	47 µg/kg
Fresh water fish	30 µg/kg

Source: (Duressa *et al.*, 2014; Bienertová-Vašků *et al.*, 2018)

In many countries, the main source of iodine is iodized salt; because it is consumed by everyone and the consumption is regular throughout years. But, mostly in developing countries knowledge , attitude and practice on iodized salt consumption is poor. Study conducted on knowledge and practice on iodized salt consumption at Debre Tabor town showed that knowledge and practice on iodized salt consumption at household level was poor. Out of 638 participants, 330 (53.8%) of the participants had poor knowledge and 341 (55.6%) had poor practice related to iodized salt consumption at household level. The factors associated with poor knowledge and practice at study area were age, marital status, educational status, occupation and source of information (Demissie, 2019). Recommended intake of iodine for different group of population (infants, children, adults, pregnant and lactating women) is different (Zimmermann, 2011).

Table 2: Recommended daily iodine intake by WHO, UNICEF and ICCIDD

Age group or population group	Recommended daily intake in µg/day
Preschool children (0-5 years)	90
School children (6-12 years)	120
Adults (>12years)	150
Pregnant women	250
Lactating women	250

Source: (Zimmermann, 2011; Agbozo *et al*., 2017; Maheswari *et al*., 2019)

Iodine has several roles in human body. It is required for synthesis of thyroid hormones (T_3 and T_4); which are important for normal growth and development of the body. Production of thyroid hormone can be decreased if there is inadequate consumption of iodine. This decreased production of thyroid hormone affects human metabolism, development and function of brain, heart, kidney and liver and it leads to IDDs like goiter, still birth, psychomotor defect and cretinism (Ahmad *et al.*, 2012). Iodine also plays essential role in promoting healthy pregnancy; during pregnancy, iodine can prevent occurrence of mental retardation in the baby. But, iodine deficiency during pregnancy can cause the baby to be born with disorder like severely stunted physical and mental growth and it may also increase the risk of miscarriage and still birth (Fardousi, 2012).

1.2.2. Thyroid gland

The thyroid gland is "butterfly" shaped gland consists of two lobes (left and right) connected by isthmus. It is located in front of the neck, below larynx (Rajabi, 2016). Thyroid gland produces, stores and releases hormones into bloodstream and direct the activity of body cells (Fardousi, 2012). It produces T_4 and T_3. The major product of the thyroid gland is T_4 (approximately 90%). Most T_3 (80%) is derived from T_4 by deiodination in peripheral tissues (liver, kidneys and muscle). The synthesis of these hormones requires the amino acid tyrosine and the trace mineral iodine. The production of these iodinated amino acids begins with the synthesis of thyroglobulin. Within thyroid gland, iodide is oxidized to iodine. This reaction is catalyzed by enzyme thyroid peroxidase. Iodine then binds to tyrosyl ring, a reaction yielding monoiodotyrosine (MIT). A subsequent addition of iodine to tyrosyl residue on MIT creates diiodotyrosine (DIT). T_4 is created by the condensation or coupling of two DIT molecules. Smaller amounts of DIT within the thyroid can also condense with MIT to form T_3 (Choksi *et al*., 2003;Setian, 2007; Šimundić *et al*., 2009; Pesce and Kopp, 2014; Rajabi, 2016).

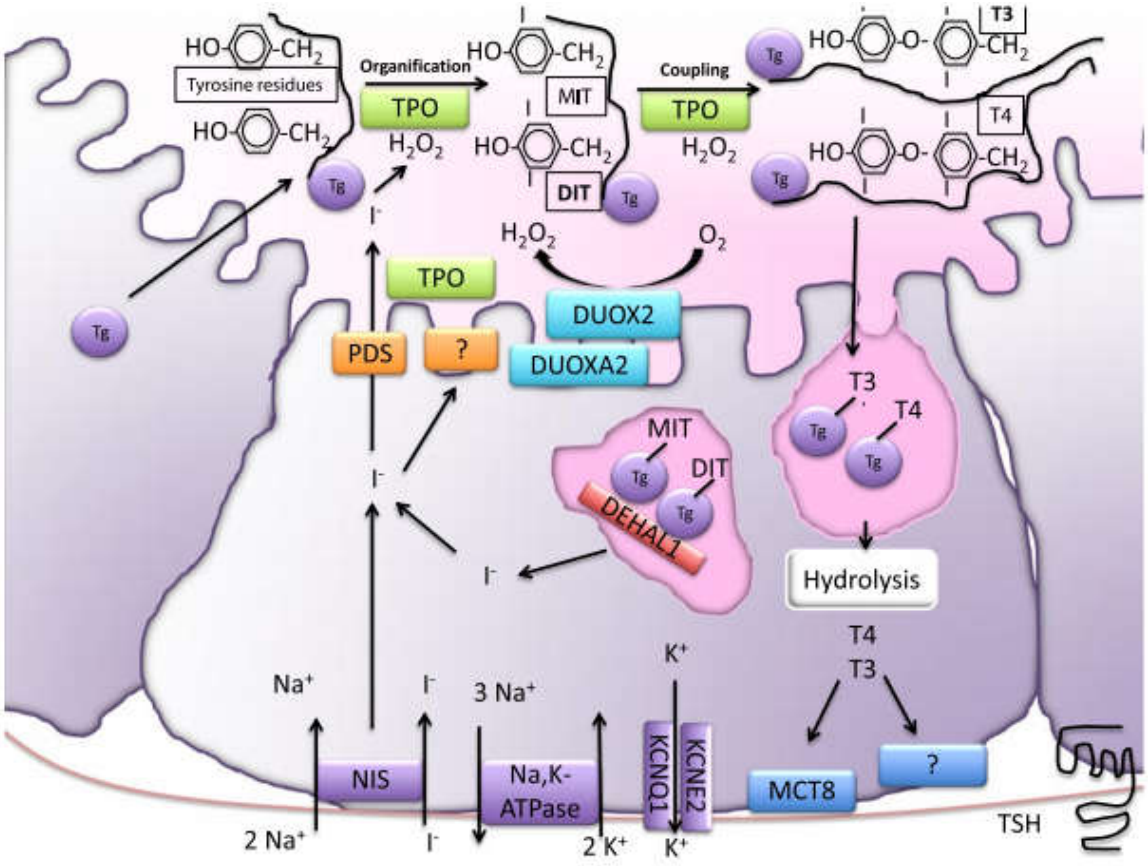

Figure 1: Mechanism of T_3 and T_4 production

The first step in iodide uptake is mediated by the sodium-iodide symporter (NIS), using the sodium gradient generated by Na, K ATPase. Active transport of potassium by the KCNE2/KCN Q1 potassium channel is also important, likely for maintaining the membrane potential of thyroid cells. At the apical membrane, pendrin (PDS) and another yet unidentified transporter mediate iodide efflux. Thyroid peroxidase (TPO), using H_2O_2 generated by the dual oxidase (DUOX2/D UOXA2) system mediates the oxidation, organification and coupling reaction that result in the sy nthesis of the iodothyronines, T4 and T3. Iodinated thyroglobulin is taken into the cell by micro and macropinocytosis and digested in lysosomes. T4 and T3 are excreted via MCT8 and other transporters. The iodotyrosines MIT and DIT are dehalogenated by dehalogenase (DEHAL1) and the released iodide is recycled (Pesce and Kopp, 2014).

The production of thyroid hormone is regulated by thyroid stimulating hormone (TSH), which is secreted by anterior pituitary gland. Thyrotropin releasing hormone (TRH), which is secreted by the hypothalamus regulates pituitary TSH secretion. Control of circulating concentration of

thyroid hormone is regulated by negative feedback loops within the hypothalamic- pituitary – thyroid axis. In general blood concentration of thyroid hormones above normal level inhibits the release of TRH and TSH. When thyroid hormone serum level is decreased, TRH and TSH release get stimulated. Increased TSH levels are associated with increased production of thyroid hormones (Hollenberg and Forrest, 2008; Fardousi, 2012).

Thyroid hormones are essential for normal development of central nervous system in infants, skeletal growth and maturation in children and normal functioning of multiple organ system in adults. Thyroid hormone stimulate metabolism in different tissues. It increases energy production and oxygen consumption in most tissues, regulate carbohydrate metabolism and stimulate the synthesis of proteins and lipolysis (Duressa *et al*., 2014)

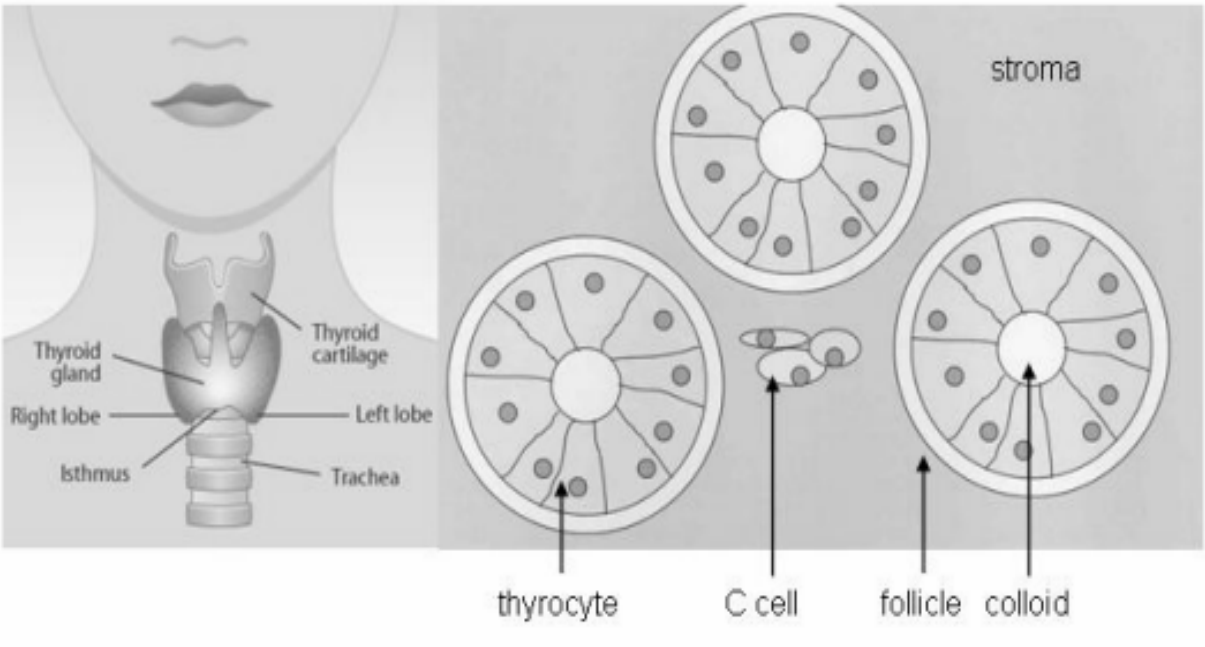

Figure 2: Image of Thyroid gland

The gland consists of thousands of follicles, each a spheroidal sac of epithelial cells (thyrocytes) surrounding a lumen containing colloid, a depot of thyroid hormone precursor, thyroglobulin (Šimundić *et al*., 2009).

1.2.3. Iodine deficiency disorder (IDD)

Iodine deficiency is associated with larger range of abnormalities called IDD, reflecting thyroid dysfunction caused by low iodine content in the diet, arising from low iodine level in the soil and water (De Benoist *et al.*, 2008; Abebe *et al*., 2017). Iodine deficiency leads to insufficient production of thyroid hormone, which have wide range of negative consequence on various organs and muscle functions, particularly heart, liver, kidney and most devastatingly in the developing of brain (Gidey *et al*., 2015).

IDD refers to a spectrum of health consequences resulting from inadequate intake of iodine or results from lack of iodine in the diet. It is one of the public health problems in many countries (Aweke *et al*., 2014). Globally, WHO estimated that 2 billion people (35.2%) of the world population are at the risk of IDD (Ambaye, 2015) and suffer from insufficient iodine intake. As a result, globally each year 22 million children are at risk of impaired intellectual function and lower school performance (Meselech Regassa *et al.*, 2016). Due to inadequate iodine in foods, drinks and aggravating factors that affect the bioavailability of iodine in the body, millions of people mainly in developing countries are affected by IDD (Aweke *et al*., 2014). It is reported that at least 350 million Africans are at risk of iodine deficiency (Agbozo *et al*., 2017). In Ethiopia it is estimated that over 35 million people are at risk of IDD and over 20 million people suffer from goiter (Arega and Asfere, 2019). The prevalence of IDD varies across regions and people living in mountainous areas. According to national survey made by Ethiopian nutrition institute, one out of every 1000 is cretin, and about 50,000 perinatal deaths are occurring annually due to IDDs (Gerensea *et al.*, 2016; Demissie, 2019). A study conducted at Tikur Anbessa Specialized Hospital showed that out of 780 patients with thyroid disease, 76.9% patients had goiter (Tsegaye and Ergete, 2003).

IDD may occur in all stage of population. IDDs include goiter, cretinism, miscarriage, severe mental retardation, deaf-mutism (Tahir *et al.*, 2016) and the like that can adversely affect the entire human body such as muscle, heart, liver, kidneys and brain (Preda *et al.*, 2013; Abebe *et al.*, 2017; Costa *et al*., 2018; Maheswari *et al.*, 2019)

Table 3: Iodine deficiency disorders by age group

Age group	Health consequence of ID
Fetus	Abortion, still birth
Neonate	Infant mortality, cretinism
Child and adolescent	Growth retardation and delayed puberty
Adult	Impaired mental function, apathy
All age	Goiter

Source: **(**Zimmermann, 2011; Pesce and Kopp, 2014)

A. Hypothyroidism

Hypothyroidism is the result of thyroid hormone insufficiency, which occurs during severe and prolonged iodine deficiency. It is caused by reduced thyroid hormone synthesis and secretion by thyroid gland. Prevalence of hypothyroidism increases with age and disorder is common in females than in males (Secretariat *et al*., 2007). Hypothyroidism is detected by low level of thyroid hormone in the blood resulting in sluggishness, depression and dry skin, loss of hair, weight gain, cold intolerance and constipation. In very young children, in addition to above symptoms it results mental and growth retardation (Assey *et al*., 2009).

B. Goiter

Goiter is outward sign of iodine deficiency and abnormal enlargement of thyroid gland (Aweke *et al.*, 2014). It indicates a condition which causes the thyroid to grow abnormally. One of the most common causes of goiter formation is iodine deficiency. The primary activity of the thyroid gland is to concentrate iodine from the blood to make thyroid hormone. The gland cannot make enough thyroid hormone if it does not have enough iodine. Therefore, individual with iodine deficiency will become hypothyroid. Consequently, the pituitary gland in the brain senses too low thyroid hormone level and sends a signal to the thyroid. This signal is called thyroid stimulating hormone (TSH). This hormone stimulates the thyroid to produce thyroid hormone and to grow in size. Thus, iodine deficiency is one cause of goiter (Duressa *et al*., 2014). At any stage goiter can affect anyone, mainly in areas where iodine rich foods are in short supply. The primary sign of goiter is swelling of thyroid gland. Other possible symptoms are the following:-

tight feeling in the throat, coughing, difficulty in swallowing and breathing (Takele *et al.*, 2003; Fardousi, 2012). Goiter is common in mountainous areas including Ethiopia, where the altitude ranges are 1,500-2,000 meters or more above sea level. In Ethiopia, it is estimated that over 35 million people are at risk of IDD and over 20 million people suffer from total goiter rate (26%). Nationally, the total percentage of goiter among school- children is 39.9%, which is the highest one among any other age groups while 36% is among biological mothers of school children (Arega and Asfere, 2019).

C. Cretinism

Iodine deficiency in critical periods of brain development and growth causes severe and permanent growth and cognitive impairment (cretinism) as thyroid hormones are required for myelination, neuronal formation and differentiation during the first trimester of gestation, and subsequently for brain growth and differentiation (Pesce and Kopp, 2014).

Cretinism is the most severe consequence of iodine dificiency occurring during fetal and neonatal life. It is the result of iodine deficiency during pregnancy, which adversely affects fetal thyroid function. For normal fetal brain development, normal concentration of thyroid hormone is very important. If there is iodine deficiency during first trimester, fetal brain may get damaged and leads to irreversible mental retardation, deaf-mutism, short stature and retarded development of musculo-skeletal system (Chen and Zou, 2004; Fardousi, 2012).

1.2.4. Causes of IDD

Many of the developing nations have the highest prevalence for IDD; it is more closely linked to populations having low income, and rural populations, who lack access of iodized food. It is common in Ethiopia because of high plateau areas. Iodine naturally found in topsoil and the topsoil has been lost due to erosion and flooding, thus crops lack iodine, resulting dietary iodine deficiency (Meselech Regassa *et al*., 2016). Therefore, lack of availability of iodine from the soil that linked to low dietary intake of iodine is the main cause of IDD. So, when the amount of iodine in food is lower than the body's need, IDD happens and consequently the thyroid gland will not be able to synthesize adequate amount of thyroid hormone.

In addition to depletion of soil iodine content, poor economic status, poor knowledge about iodized salt (Anteneh *et al*., 2017), adding salt during or at the beginning of food preparation and poor knowledge about consequence of iodine deficiency (Arega and Asfere, 2019) are significantly associated with iodine deficiency. Furthermore, there are factors that increase risk of developing iodine deficiency such as use of unpacked salt (study conducted in Dera District, Northwest Ethiopia showed that salt samples stored in a closed containers were 1.7 times more likely to have adequate iodine content compared to salt samples stored in an open containers (Anteneh *et al*., 2017), storing salt for a long duration and exposing to heat and sunlight (Abebe *et al.*, 2017).

Goitrogen is another risk for iodine deficiency. It is a substance that interferes with iodine utilization. Goitrogen is metabolized to thiocyanate and blocks thyroidal uptake of iodine. Some species of millet and cruciferous vegetables contain goitrogen. In addition, some plants such as cassava contain thiocyanate that inhibits thyroid iodide transport (Aweke *et al*., 2014; Ershow *et al*., 2018).

1.2.5. Prevention and control of IDD

When there is lack of iodine in the earth crust, iodine deficiency happens. Naturally iodine does not occur in specific foods; when iodine is lost by erosion from the soil, the crops that grow in that soil lacks iodine. As a result, crops do not provide sufficient dietary iodine to the population. So, soil erosion should be prevented by using different techniques (Ahmad *et al*., 2012).

Many people do not know how to use and store iodized salt in proper manner (Abebe *et al*., 2017). Iodized salt should be used after cooking; do not use during cooking. Iodine may sublime to the atmosphere because of heat when iodized salt is added during cooking. The level of post-production loss of iodine at the seller and consumer level can be reduced by storing of salts in (cool and dry place, clean air tight plastic container) and avoiding excess exposure to sunlight or heat (Gidey *et al*., 2015).

Monitoring of iodine content in the salt at the production and import sites before salt enters into local markets to guarantee correct and safe iodine level is reaching to consumers and taking legal action against noncompliant salt producers or traders is the main thing to prevent and control IDD (Takele *et al*., 2003).

IDDs cannot be eliminated by simply changing dietary habits or by eating specific kinds of foods. The recommended strategy for IDD control is based on correcting the deficiency by increasing iodine intake through supplementation or food fortification (Aweke *et al.*, 2014).

1. Salt iodization

Salt iodization involves the addition of predetermined amount of iodine to the salt during production process to ensure the correct dosage of iodine as stipulated by government standard. It is first line public health measure for preventing and controlling iodine deficiency disorders (Peterson, 2000; Fardousi, 2012).

There are two salt fortificants; iodate and iodide, which are usually added as potassium salt. Potassium iodate (KIO_3) has 59.5% iodine, while potassium iodide (KI) contains 76.5% iodine. KI is more soluble in water and less expensive than KIO_3, but under condition of moisture, exposure to sunlight or heat, KI is less stable than KIO_3. Therefore, iodate is more resistant to oxidation and evaporation. To avoid oxidation of iodide with atmospheric oxygen the iodization of edible salt is done by adding iodate instead of potassium iodide (Khazan *et al.*, 2013; Nepal *et al.*, 2013; Rajabi, 2016).

Universal Salt Iodization (USI) is the primary recommended strategy to prevent IDD and it was adopted in 1993. USI is the iodization of salt in food industry and household for human consumption in order to control IDD. In 1994 Special section of WHO, UNICEF and ICCIDD joint committee on health policy recommended USI as safe, cost effective and sustainable strategy to ensure adequate intake of iodine by all individuals (WHO, 2007; Gidey *et al*., 2015; Maheswari *et al*., 2019)**.**

There is no universal specification for the level of iodine to be added to salt to achieve recommended intake of iodine. The appropriate level of salt iodization depends on the individual consumption of salt, the degree of iodine deficiency in the area and the loss of iodine from producer to consumer. All the above factors may not be the same in different countries; so, the levels of salt iodization vary substantially between different countries and over time. For example, the Brazilian legislation claims that iodine concentration in table salts should be within the range of 15 to 45 mg/kg (Costa *et al*., 2018). According to New Zealand Food Safety Authority's, iodized salt must contain 25-65 milligrams of iodine per kilogram of salt (Thomson,

2009). In Indonesia the content of iodine in table salt must be between 30 and 80 ppm as potassium iodate (Wulandari and Rosyida, 2017). According to WHO, UNICEF and ICCIDD, the salt has to be fortified with 20 – 40 mg of iodine per kilogram of salt at the production site and at the household level it should have 15-40 ppm of iodine (Ebrahim and Muhammed, 2012; Gerensea *et al*., 2016; Maheswari *et al*., 2019).

(Note: (mg/kg = ppm) so it can be used interchangeably)

In Ethiopia USI program was launched in 1995/1996. In June 2004 the National Guideline for Control and Prevention of Micronutrient Deficiency formulated by the Ethiopian Federal Ministry of Health (EFMH) states that in Ethiopia, iodine content of salt to be 80 - 100 ppm as KIO_3 at the port of entry. Quality and Standard Authority of Ethiopia (QSAE) has set the iodine level to be 60 - 80 ppm as KIO_3 or 36 - 48 ppm as iodine after making allowance for losses of iodine during storage and distribution (Gerensea *et al*., 2016). According to salt iodization council of ministers regulation number 204/2011, no person shall process, import, store, transport, distribute or sale non iodized salt for human consumption. Any iodized salt for human consumption shall conform with the standards for iodized salt set by the appropriate authority and any iodized salt shall be labeled with the following information on the package; iodine content of the iodized salt, the name and complete address of the processor, net weight of the iodized salt, month and year in which the iodized salt is processed and expired, batch and lot number of the package and description that the salt shall be kept in a cool, clean and dry place. Any person who violates the provisions of this regulation or directives issued for the implementation of this regulation shall be punished in accordance with the relevant provisions of the proclamation (Yohannes, 2011). The average consumption of salt per adult person per day is 10 grams and salt with iodine content of 60 mg/kg would satisfy the recommended daily requirement of 150µg of iodine per adult to prevent IDD (Takele *et al*., 2003; Gerensea *et al*., 2016).

1.2.6. The risk of excess iodine intake

The excessive intake of iodine may cause over stimulation of the thyroid gland, which produce excess hormone and cause hyperthyroidism (Costa *et al*., 2018). As iodine deficiency impairs thyroid function, iodine excess including over correction of previous state of iodine deficiency

can also impair thyroid function. Both high and low iodine intakes are associated with increased risk of thyroid disorders (Leung and Braverman, 2014).

1.2.7. Methods for determining iodine content in the salt

Recently, several methods for testing the iodine content of salt has been reported ranging from qualitative spot test by rapid test kit to the more quantitative methods such as spectrometric, iodometric titration, electrochemical methods and the like (Rohner *et al.*, 2015; Rajabi, 2016). In this study rapid test kit and iodometric method were chosen and used because of reasons discussed below.

1. Rapid test kit

Rapid test kit (RTK) is small 10-50 ml bottle containing a starch, acidic buffer and KI solution. When the solution is dropped on to a sample of salt containing iodine a blue or purple stain develops, indicating the presence of iodine. The intensity of the blue color indicates the iodine concentration in the salt and color charts have been developed to facilitate the 'reading' or classification of the iodine content. RTK have been widely used to assess the presence and, in some cases, the adequacy of iodine in salt (WHO, 2007; Gorstein *et al*., 2016). The reason for choice of RTK method in this study is because of its simplicity and it does not require any infrastructure, is inexpensive, and most importantly, it provides immediate results suitable for rapid feedback. The kits give either a qualitative or a semi quantitative estimation of the iodine content. Qualitative means that salt samples are classified as (adequately) iodized or uniodized. The test gives no indication of the actual level of iodine in the salt. Some kits give a semi quantitative estimate of iodine content of the salt. For example, one kit categorizes salt samples as having an iodine content of 0, < 15, or >15 ppm (Pandav *et al*., 2000; Rajabi, 2016).

2. Iodometric titration

It is analytical method used to assess iodine content in salts quantitatively and required to monitor the adequacy of salt iodization at the level of production, importation and consumption. Iodometric method requires laboratory equipment and skilled personnel to handle the methodology correctly (Ranganathan *et al*., 2015; Rohner *et al.*, 2015; Yadav *et al*., 2015). It is the most commonly used method, still remains reference method for determining iodine content

in the salt (Dearth-Wesley *et al*., 2004; Yadav *et al*., 2015). When other methods are used, it should be standardized against titration method. It is preferable to quantify iodine content of salt accurately (Guamuch *et al.*, 2007; WHO, 2007; Khazan *et al.*, 2013). Iodometric titration is the most accurate "gold standard" analytical method recommended for factory quality control and assurance, and research studies (Yadav *et al*., 2015; Myers *et al*., 2016; Moyib, 2018). Many studies expressed and agreed that titration method is the one mostly used worldwide in the determination process of iodine quantity in iodized salt. This method is chosen due to its accuracy, precision, ease of operation and low cost (Khazan *et al*., 2013; Ranganathan *et al.*, 2015; Rohner *et al.*, 2015; Rajabi, 2016).

1.3. Statement of the problem

On the earth iodine has uneven distribution, it is found in water (lake, seawater and ocean) and in the soil (Meselech Regassa *et al.*, 2016). Mainly marine water bodies (ocean and sea water) contain higher concentration of iodine. Therefore, marine foods (plants and animals) have higher concentration of iodine and used as the main source of iodine, since marine plants and animals concentrate iodine from seawater or ocean (Zimmermann, 2011). But in Debre Tabor, there is no seawater or ocean; therefore, the people in Debre Tabor do not get iodine from marine plants and animals.

In many areas of the world, the soil does not contain adequate amount of iodine. Especially, in mountainous areas like Himalayas, Andes and Alps, soil has inadequate amount of iodine leading to the prevalence of IDD. Central Africa, Central Asia and much of Europe also have iodine depleted soil (Zimmermann *et al.*, 2008; Rajabi, 2016). So, iodine deficient soils are common in mountainous area and areas having frequent flooding (Pesce and Kopp, 2014) like Ethiopia (mainly northern part of Ethiopia). In Ethiopia iodine content of water and soil is low and the iodine level of non-farm land in plain land is higher than hilly land (Mohammed, 2016). Iodine is found naturally in topsoil but in most areas of the country and especially the highlands, top soil has been lost due to deforestation, erosion and flooding, and foods that grow on iodine depleted soil lack iodine resulting in dietary iodine deficiency (Duressa *et al*., 2014). Since the landscape of Debre Tabor and its surrounding is hilly and there is frequent flooding, as a result, different minerals including iodine may be eroded with soil. For this reason, locally cultivated foods may not contain adequate amount of iodine; besides these there is an indication of iodine deficiency due to the presence of people with goiter, impaired mental function and growth retardation. Study conducted on assessing prevalence of goiter and its associated factors among primary school age children aged 6-12 years old in Debre tabor town, northwest Ethiopia showed that out of 289 children 82 (28.37%) had goiter (Arega and Asfere, 2019).

1.4. Significance of the study

In developing countries, salt production and the quality of available salt may be poor or salt may be incorrectly iodized or salt that has been correctly iodized may deteriorate due to excessive or long term exposure to moisture, light or heat (Abebe *et al*., 2017). All these factors should be taken carefully into account, particularly when establishing the initial level of iodine in salt (production level), at shop and consumption level.

Many people, mainly from developing countries are under the risk of IDD. These IDDs include goiter, cretinism, deaf mutism, increased still birth and abortion (Ahmad *et al*., 2012). So, these disorders can be successfully prevented by correction of iodine deficiency by consumption of adequate amount of iodine in the diet. USI is a recommended intervention for preventing and correcting iodine deficiency. Prevention can result in improved quality of life, productivity, and educability of children and adults (Fardousi, 2012).

The aim of this study was to determine iodine content of salts commercially available for human consumption collected from the shop in Debre Tabor town using iodometric titration and RTK. So, this study will give baseline information for concerned bodies involved in controlling and monitoring the iodine content of salts available for human consumption.

2. OBJECTIVE

2.1. General objective

- To determine iodine content in salt samples commercially available in Debre Tabor town, using Rapid test kit and iodometric titration

2.2. Specific objectives

- To determine iodine content of salts collected from Debre Tabor town
- To compare iodine content between different salt brands
- To compare level of iodine in salts with WHO and Ethiopian standard

3. EXPERIMENTAL METHODS

3.1. Equipment and Materials

The equipment and materials used in this research were beaker, hot plate, measuring cylinder, volumetric flask, glass rod, electronic balance, graduated pipette, burette, stand, Erlenmeyer flask, aluminum foil, watch glass, and white cup.

3.2. Chemicals and Reagents

Potassium iodate (chemicals UDYOG, India), Sodium thiosulfate (Alpha chemika, India), Sulfuric acid (Sigma Aldrich, Germany), Potassium iodide (chemicals UDYOG, India), Starch (Avi chem industry, India), Iodine rapid test kit solutions (MBI chemicals, India), and distilled water (Fardousi, 2012; Ajmal *et al*., 2018). All the chemicals and reagents used were of analytical grade.

3.3. Description of study area

The study was conducted at Debre Tabor Town, South Gondar that is located 103 km away from Bahir Dar and 666 km away from the capital city of Ethiopia, Addis Ababa. It is located about 100 km South East of Gondar and 50 km East of Lake Tana. The town is located in Amhara regional state in the Northern part of Ethiopia and its elevation is 2706 meters above sea level.

3.4. Study design

Cross-sectional study was conducted to determine the iodine content in salt samples commercially available in Debre Tabor town within study period (from December 16 to 29, 2019).

3.5. Eligibility criteria

- ✓ Salts having three months and above of shelf life left before expiry date were selected for analysis.

3.6. Sample collection

Training was given for sample collectors about the environmental factors that affect the availability of iodine in iodized salt. Different salt brands were purchased from retail shops by twelve trained sample collectors in proper manner. The salt packets were checked for proper sealing and quality of packing before buying, as there are chances of iodine loss by oxidation in open air. Totally, 27 salt packets were purchased. Out of which, 10 salt packets (fine - 9 and coarse- 1) were included for the study, excluding some salt packets of same brand. Salt samples were kept in the dark until analysis and the anonymity of salt samples was distinguished by the name on the cover or pack.

Table 4: Description of samples

Type of salt sample	Sample name	Labeled iodine value in (mg/kg)	Production date	Expiry date	Manufactured in
Fine packed salt	Risa	15-40	07/ 2019 GC	07/ 2022 GC	Saudi Arabia
	MTI	NG	01/ 02/ 2012 EC	01/ 02/ 2013 EC	Ethiopia
	Fayadhaa	NG	07/ 2017 GC	07/ 2021 GC	Ethiopia
	Shega	NG	04/ 2019 GC	04/ 2021 GC	Ethiopia
	Edeget	NG	03/ 2018 GC	03/ 2020 GC	Ethiopia
	Afar	NG	10/ 04/ 2019 GC	11/ 04/ 2020 GC	Ethiopia
	Safe	NG	01/ 08/ 2019 GC	01/ 08/ 2021 GC	Ethiopia
	Waliya	NG	01/ 01/ 2012 EC	01/ 01/ 2013 EC	Ethiopia
	Woff	30-40	28/ 10/ 2019 GC	27/ 10/ 2021 GC	Ethiopia
Coarse packed salt	SVS	NG	04/ 2012 EC	04/ 2014 EC	Ethiopia

NG = Not given, GC = Gregorian calendar, EC = Ethiopian calendar

3.7. Data quality control and assurance measurements

Great emphasis was given to data quality control and assurance starting from data collection, laboratory testing, data entry and statistical analysis. The laboratory reagents and the instruments used were of standard quality. Salt samples were taken from top, middle and bottom for each salt packet because iodine content may vary in samples taken from the same container of different depth (top, middle, and bottom). Laboratory analysis of all samples was performed in triplicates. Special emphasis was paid on data entry to software and the final result was checked repeatedly to assure overall data quality.

3.8. Preparation of standard solution and reagents

0.005N Sodium thiosulfate: weighed mass of 1.24 g $Na_2S_2O_3.5H_2O$ was placed into one liter volumetric flask. Then distilled water was added in small amount and shaken until solute dissolves. Finally, distilled water was filled up to 1 liter mark.

2N sulfuric acid: 8 ml of concentrated H_2SO_4 was added to 100 ml of distilled water and made up to 150 ml with water.

10% potassium iodide: 100 g of potassium iodide was weighed and transferred into 1000 ml volumetric flask. After that distilled water was added to flask in small amount and the solution was shaken until solute dissolves. Then distilled water was filled up to 1000 ml mark and stored in cool, dark place.

1% Starch solution: weighed mass of 1 g of starch was transferred into 100 ml beaker. Then distilled water was added up to 100 ml mark and heated to dissolve.

0.01N standard potassium iodate solution: 0.018 gram of standard potassium iodate was accurately measured and placed into 50 ml volumetric flask. Distilled water was added to flask in a small amount and shaken until the solute dissolved. Then flask was filled with distilled water, up to 50 ml mark**.**

3.9. Standardization of sodium thiosulfate solution

The sodium thiosulfate solution should be standardized with standard potassium iodate solution to determine its exact normality.

15 ml of 0.01N KIO_3 solution was pipetted out in to 250 ml Erlenmeyer flask and 1 ml of 2N sulfuric acid and 5 ml of 10 % potassium iodide solution were added. Then flask was covered with aluminum foil and kept in the dark for 10 minutes. After specified time, flask was taken out of dark and titrated against sodium thiosulfate till the solution became pale yellowish in color. Then 2 drops of starch solution was added and the solution turned blue black. Finally, solution was titrated again with thiosulfate solution till the blue black color completely disappears. And then process was continued two more times and the average volume of thiosulfate was recorded to calculate exact concentration of thiosulfate.

Table 5: Standardization of sodium thiosulfate

Number of trials	Volume of thiosulfate consumed in (ml)	Average in (ml)
1	29.9	29.93
2	30.1	
3	29.8	

Normality of sodium thiosulfate is calculated using the following formula:

$V1*N1 = V2*N2$----------1

Where,

V1- volume of potassium iodates = 15 ml

N1- normality of potassium iodate = 0.01 N

V2- volume of sodium thiosulfate = 29.93 ml

N2- normality of sodium thiosulfate =?

So, $N2 = (V1*N1)/V2$----------2

$N2 = (15 ml * 0.01N)/29.93 ml$

$N2 = 0.005 N$

3.10. Validation of Iodometric and RTK methods for testing iodine in the salt

Titration method is recommended for the correct determination of the amount of iodine present in salt. Randomly Waliya salt was selected and iodine content of Waliya was measured ten times using iodometric titration and its average iodine concentration was used as reference and again Waliya salt was tested ten times using RTK to confirm positivity and negativity with iodinated and non-iodinated salt, respectively. So each method was used to measure iodine level in different salt brands. Iodometric titration is the most commonly used method, still remains reference method for determining iodine content in the salt. When other methods are used, it should be standardized against titration method (Guamuch *et al*., 2007; Khazan *et al*., 2013).

3.11. Principle and Procedure for use of rapid Testing Kit

Principle

The estimation of the salt iodine by using RTK is based on the reaction between starch and iodine to form starch-iodine complex. RTK solution contains an acidic buffer, starch and reducing agent, which convert potassium iodate to iodine. Iodine liberated from salt or potassium iodate forms visible blue color with starch depending on the amount of iodine in the salt. The color of the sample is compared with the standard color chart for determining the salt iodine content (Diosady *et al*., 1999; Nepal *et al*., 2013).

$$IO_3^- + 5\,\mathrm{I}^- + 6H^+ \longrightarrow 3I_2 + 3\,\mathrm{H_2}\,O \text{ ----------1}$$

$$3I_2 + \text{Starch} \longrightarrow \text{blue colored starch iodine complex ----------2}$$

Procedure

Small cup was filled with salt and the salt surface was spread flat. Then two drops of the test solution was added on the surface of the salt. After that the color on the salt was developed and color on the salt was compared with the standard color chart, and iodine content was determined. Finally, the procedure was continued two more times. If no color appears on the salt after one minute, on fresh sample add up to five drops of recheck solution and then add two drops of test solution on the same spot. Finally, compare the sample color with standard color chart and determine the iodine content (Meselech Regassa *et al*., 2016).

Photograph of standard color chart of RTK and color of Risa salt before and after addition of RTK solution

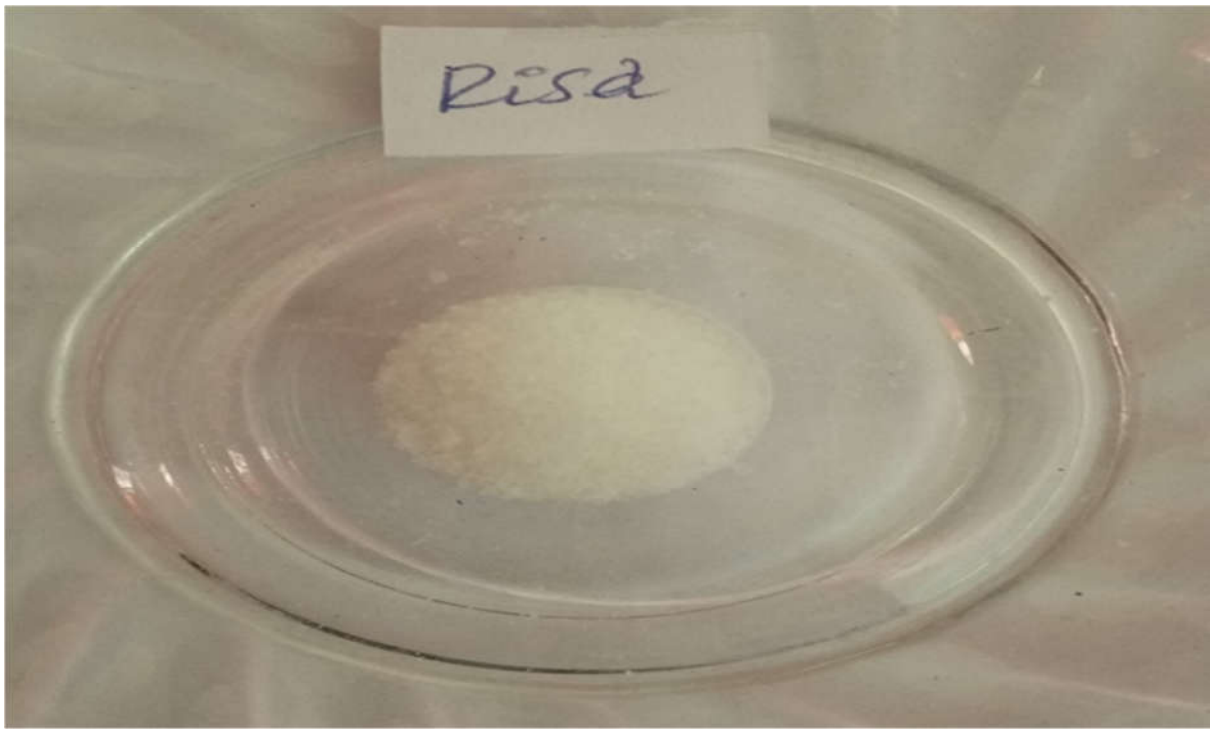

Figure 3: Photograph of Risa salt before RTK solution is added

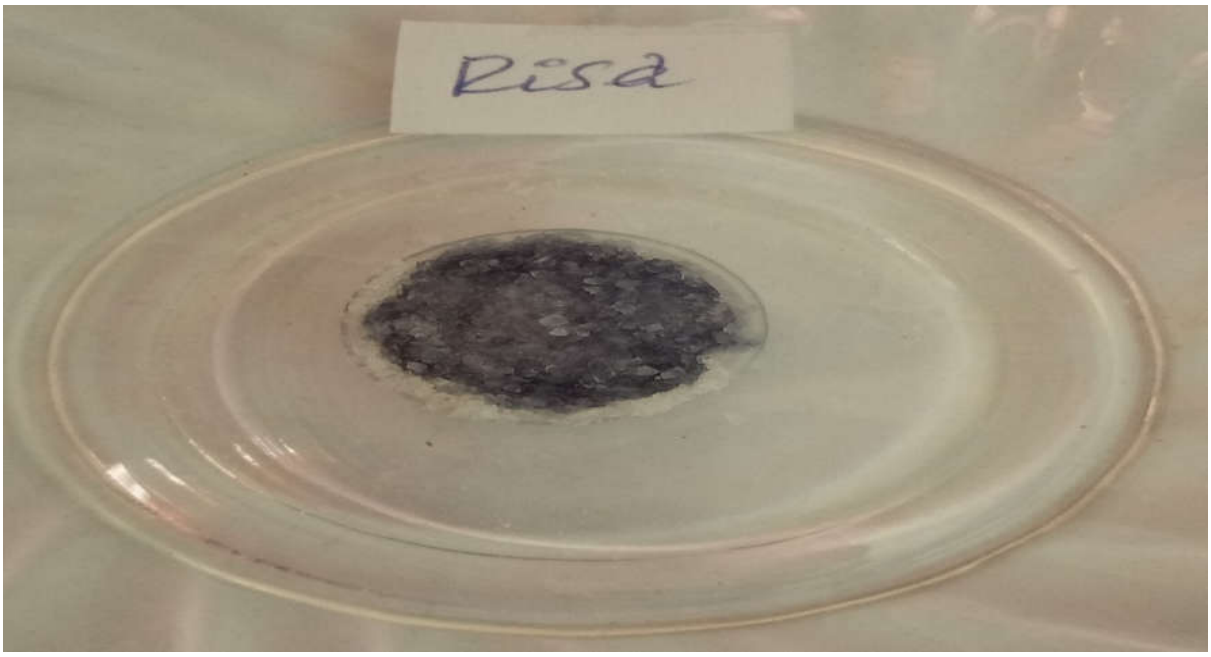

Figure 4: Color of Risa salt after RTK solution is added

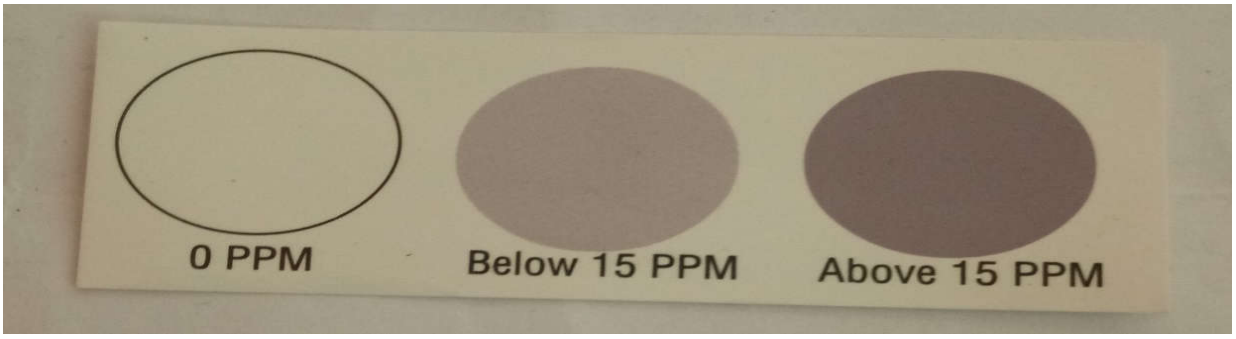

Figure 5: Standard color chart of RTK

3.12. Principle and Procedural steps for use of iodometric titration

Principle

Most salts are fortified with potassium iodate (KIO_3). To determine iodine added as iodate, salt is dissolved in slightly acidic solution to which excess potassium iodide (KI) is added. The iodate from the salt reacts with iodide (I^-) to form iodine (I_2) and the solution turns to yellow color depending on the amount of iodine liberated. Iodine liberated is titrated with sodium thiosulfate, until the solution turns to pale yellow. Starch solution is added as indicator of this reaction, and it reacts with the free iodine to produce a blue color when it is added towards the end of the titration (when only a trace amount of free iodine is left). Finally, free iodine is consumed by sodium thiosulfate in the titration step and end point is visually determined by disappearance of blue or blue black color from solution when no more iodine is present (Nepal *et al.*, 2013).

Related chemical equations are as follows:-

Liberation of iodine from the iodate in salt solution

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O \quad \text{----------1}$$

Reduction of iodine with thiosulfate

$$I_2 + 2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2I^- \quad \text{----------2}$$

Overall reaction

$$IO_3^- + 5I^- + 6H^+ + 6S_2O_3^{2-} \longrightarrow 3S_4O_6^{2-} + 6I^- + 3H_2O \quad \text{----------3}$$

In iodometric titration, the amount of iodine liberated from salt is proportional to the amount of thiosulfate used during titration and the amount of iodine in salt is calculated by the following formula:

Iodine in mg/kg = $\frac{V \times 0.001 \times N \times 1000 \times 21.22}{M}$ ----------4

Where,

V = Average volume of $Na_2S_2O_3$ in milliliter

0.001 = Conversion factor to convert milliliter of $Na_2S_2O_3$ to liter

N = Normality of $Na_2S_2O_3$ which is 0.005 equivalent per liter

M = Mass of salt sample which is 0.01 kilogram or 10 gram

1000 = conversion factor to convert gram of iodine to milligram

21.22 = Equivalent weight of iodine in gram per equivalent

(Rohner *et al*., 2015; Mizehoun-Adissoda *et al*., 2018)

Procedural steps

A. Solubilization of salt samples

A weighed mass of 10 g of salt sample was placed into 250 ml Erlenmeyer flask and 50 ml of distilled water was added to dissolve salt. Then 1 ml of 2N H_2SO_4 and 5 ml of 10% KI were added to salt solution and the solution turned to yellow as iodine was produced. Finally, the flask was covered with aluminum foil and put in the dark for 10 minutes (Mizehoun-Adissoda *et al*., 2018)

B. Titration

Rinsed burette was filled with 0.005N $Na_2S_2O_3$. After specified time, the flask containing solution was taken from the dark, and some thiosulfate was added from titration burette with continuous gentle agitation until yellow solution became pale yellow. Then, 2 drops of starch solution was added and the solution was turned to blue-black color. Titration was continued until the solution color turned to colorless (Nepal *et al*., 2013; Mizehoun-Adissoda *et al*., 2018). Finally, titration was repeated two more times and average volume of $Na_2S_2O_3$ was determined.

Photograph of color changes in iodometric titration for Waliya salt

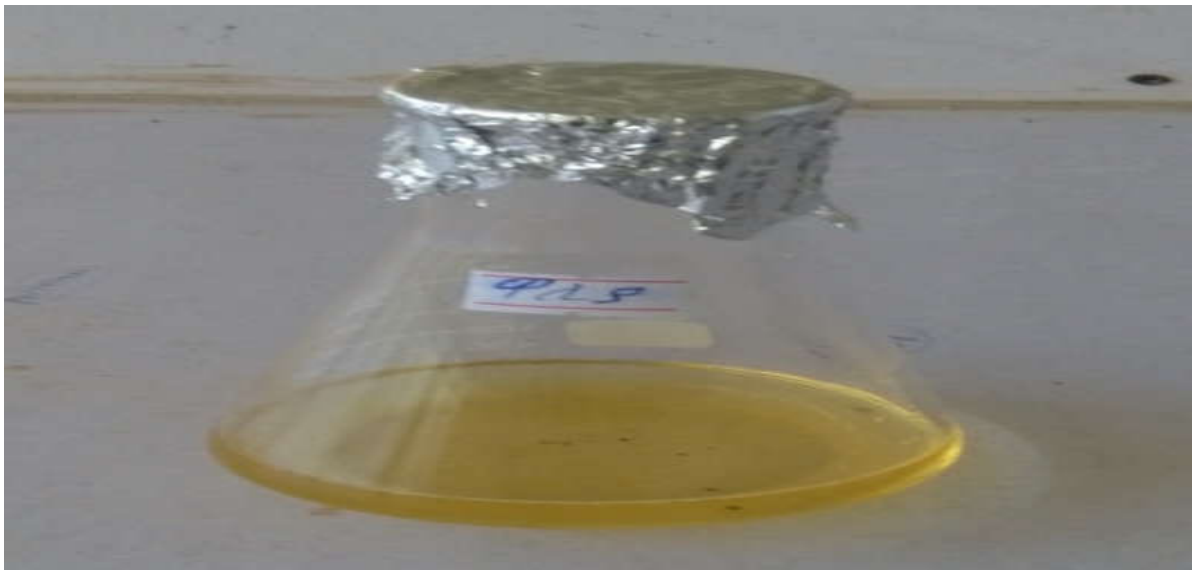

Figure 6: Yellow color of iodine formed from the reaction of iodized salt with acidic iodide solution

Figure 7: Pale yellow color left when nearly all iodine has reacted with thiosulfate during titration

Figure 8: Blue black color formed when starch indicator is added to pale yellow solution

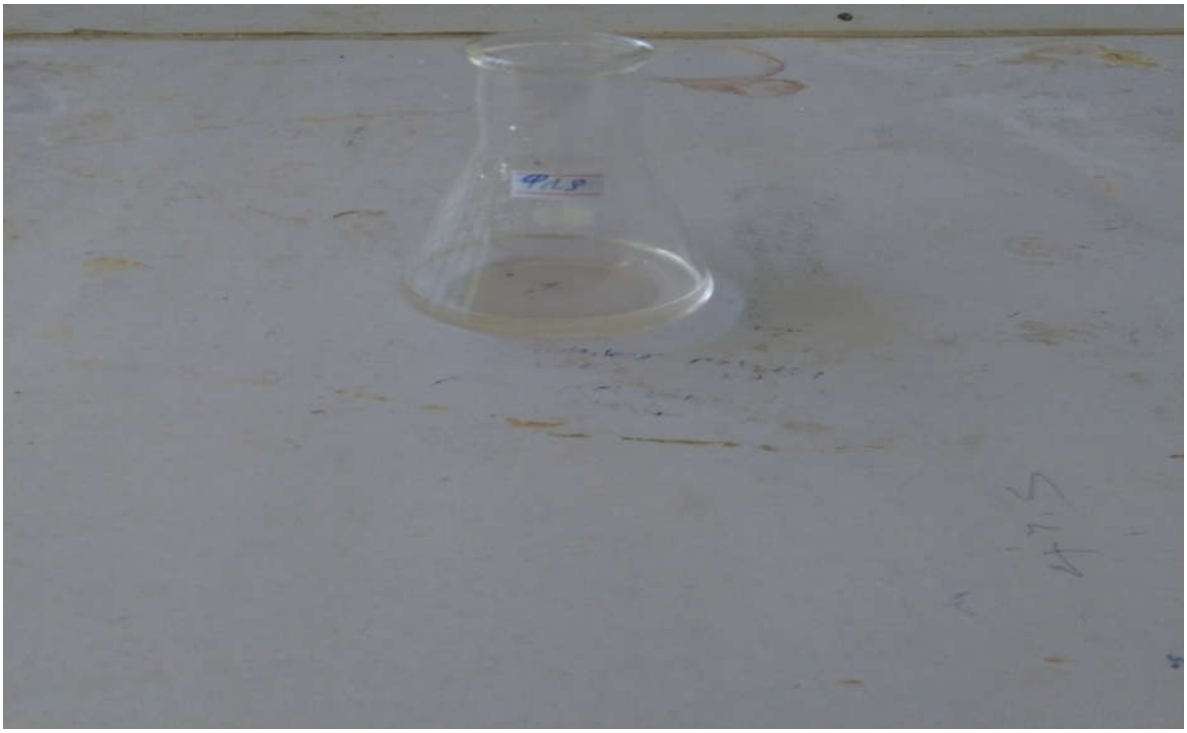

Figure 9: Blue black color disappeared, leaving colorless solution at the end point

3.13. Data analysis

All statistical analyses were analyzed using the software, Statistical Package for the Social Science (SPSS) version 21 and results were expressed as mean ± standard deviation. Statistical difference between mean iodine content of salt was analyzed by One-Way Analysis of Variance (ANOVA) and statistical significance was accepted at $p < 0.05$.

4. RESULT AND DISCUSSION

4.1. Iodine content of salt samples collected from shops of Debre Tabor town in 2019

Following the procedure, as mentioned above the following results were obtained.

Table 6: Iodine content of salt samples collected from shops of Debre Tabor town in 2019

Type of salt sample	Sample name	Iodine content by	
		Iodometric titration (mean ± standard deviation) in (mg/kg)	Rapid test kit in (mg/kg)
Fine packed salt	Risa	37.252 ± 0.603	>15
	Shega	19.57 ± 0.167	>15
	Fayadhaa	17.449 ± 1.016	>15
	Safe	31.123 ± 0.29	>15
	Waliya	20.984 ± 0.441	>15
	Afar	26.643 ± 0.727	>15
	MTI	28.53 ± 0.728	>15
	Ediget	11.435 ± 0.441	<15
	Woff	24.875 ± 0.727	>15
Coarse packed salt	SVS	25.814 ± 0.866	>15

As seen in **Table 6,** the iodine content of different salt samples commercially available in Debre Tabor town was determined by iodometric titration and RTK. The iodometric titration results showed that iodine content of salt samples was found within the range of 11.435 ± 0.441mg/kg to 37.252 ± 0.603mg/kg. This indicates that, salt samples available in the study area show non-homogenous distribution of iodine in the salt; that is iodine content in Risa (37.252± 0.603mg/kg), Shega (19.57 ± 0.167mg/kg), Fayadhaa (17.449 ± 1.016mg/kg), Safe (31.123 ± 0.29mg/kg), Waliya (20.984 ± 0.441mg/kg), Afar (26.643 ± 0.727mg/kg), MTI (28.53 ± 0.728mg/kg), Ediget (11.435 ± 0.441 mg/kg), Woff (24.875 ± 0.727mg/kg), and SVS (25.814 ± 0.866mg/kg). Iodine content (30 - 40 mg/kg) leveled on package of Woff salt was not confirmed

during analysis (24.875 ± 0.727mg/kg). This difference or variability of iodine content in different salt brands commercially available for human consumption in the Debre Tabor town and lack of conformity of labeled iodine (30 - 40mg/kg) value on package of Woff salt during analysis may be due to non-homogenous distribution or variability of iodine amount added during iodization and poor mixing resulting uneven distribution within bags (batches), exposure to high temperature and moisture during distribution, transportation and that may be due to long exposure to moisture while working in laboratory, inaccuracy and oversight due to faulty equipment at production level or factory and duration of salt storage at production or shop level, and it may be because of production date difference and the difference in the type or quality of container or packet that the salt is packed. Study conducted on determination of iodine content of dietary salt at household level and associated factors using iodometric titration methods in Dera District, Northwest Ethiopia showed that Salt samples stored for less than 2 months were 1.6 times more likely to have adequate iodine content compared to those salt samples stored for over 2 months (Anteneh *et al*., 2017). Another study conducted in London showed that duration of salt storage had impact on the level of iodine. Iodized salt will lose 24% iodine when stored for 10 weeks. This may be due to the effect of environmental factors like moisture content of salt, light, heat and other weather conditions. Similar study conducted in Colombia showed effect of longer storage beyond two months aggravated loss of iodine from salt due to environmental conditions during storage and distribution (Gebremariam *et al*., 2013). A study conducted for determination of iodine level in consumer table salt from production to consumption in Palestine, by studying the relationship between the date of production and the percentage loss; found that the iodine loss increased as a result of shelf time increase for most salt brands (Rajabi, 2016). But this study showed that relationship between date of production and loss of iodine content is not proportional as shelf life increases for most of salt brands. The reason behind production date and loss of iodine disproportionality for most of salt brands as shelf life increases may be due to long exposure to high temperature and moisture during distribution, transportation and storage, and it may be due to difference in the quality of container that the salt is packed with. The salt iodine content determined by the RTK method showed that all salt brands have iodine content greater than 15 ppm except Ediget salt (< 15 ppm). The reason for this may be because of exposure of similar factors which are listed above and may be because of difference in shelf life, which are left to expire.

4.2. Statistical comparison of iodine content between different salt brands collected from different shops

Table 7: Comparison of iodine content between different salt brands collected from different shops of Debre Tabor in 2019

Salt sample and its iodine content in mg/kg		P-value	Salt sample and its iodine content in mg/kg		P-value
SVS (25.814±0.866)	Risa (37.252 ± 0.603)	0.000*	Ediget (11.435±0.441)	Risa (37.252 ± 0.603)	0.000*
	Shega (19.57 ± 0.167)	0.000*		Shega (19.57 ± 0.167)	0.000*
	Fayadhaa (17.449±1.016)	0.000*		Fayadhaa (17.449±1.016)	0.000*
	Safe (31.123 ± 0.29)	0.000*		Safe (31.123 ± 0.29)	0.000*
	Waliya (20.984±0.441)	0.000*		Waliya (20.984±0.441)	0.000*
	Afar (26.643 ± 0.727)	0.947		Afar (26.643 ± 0.727)	0.000*
	MTI (28.53 ± 0.728)	0.013*		MTI (28.53 ± 0.728)	0.000*
	Ediget (11.435±0.441)	0.000*		Woff (24.875± 0.727)	0.000*
	Woff (24.875± 0.727)	0.901		SVS (25.814±0.866)	0.000*
Woff (24.875±0.727)	Risa (37.252 ± 0.603)	0.000*	MTI (28.53 ± 0.728)	Risa (37.252 ± 0.603)	0.000*
	Shega (19.57 ± 0.167)	0.000*		Shega (19.57 ± 0.167)	0.000*
	Fayadhaa (17.449±1.016)	0.000*		Fayadhaa (17.449±1.016)	0.000*
	Safe (31.123 ± 0.29)	0.000*		Safe (31.123 ± 0.29)	0.020*
	Waliya (20.984±0.441)	0.000*		Waliya (20.984±0.441)	0.000*
	Afar (26.643 ± 0.727)	0.232		Afar (26.643 ± 0.727)	0.171
	MTI (28.53 ± 0.728)	0.001*		Ediget (11.435±0.441)	0.000*
	Ediget (11.435±0.441)	0.000*		Woff (24.875± 0.727)	0.001*
	SVS (25.814±0.866)	0.901		SVS (25.814±0.866)	0.013*

Salt sample and its iodine content in mg/kg		P-value	Salt sample and its iodine content in mg/kg		P-value
Afar (26.643± 0.727)	Risa (37.252 ± 0.603)	0.000*	Waliya (20.984±0.441)	Risa (37.252 ± 0.603)	0.000*
	Shega (19.57 ± 0.167)	0.000*		Shega (19.57 ± 0.167)	0.505
	Fayadhaa (17.449±1.016)	0.000*		Fayadhaa (17.449±1.016)	0.001*
	Safe (31.123 ± 0.29)	0.000*		Safe (31.123 ± 0.29)	0.000*
	Waliya (20.984±0.441)	0.000*		Afar (26.643± 0.727)	0.000*
	MTI (28.53 ± 0.728)	0.171		MTI (28.53 ± 0.728)	0.000*
	Ediget (11.435±0.441)	0.000*		Ediget (11.435±0.441)	0.000*
	Woff (24.875± 0.727)	0.232		Woff (24.875± 0.727)	0.000*
	SVS (25.814±0.866)	0.947		SVS (25.814±0.866)	0.000*
Safe (31.123 ± 0.29)	Risa (37.252 ± 0.603)	0.000*	Fayadhaa (17.449±1.016)	Risa (37.252 ± 0.603)	0.000*
	Shega (19.57 ± 0.167)	0.000*		Shega (19.57 ± 0.167)	0.088
	Fayadhaa (17.449±1.016)	0.000*		Safe (31.123 ± 0.29)	0.000*
	Waliya (20.984±0.441)	0.000*		Waliya (20.984±0.441)	0.001*
	Afar (26.643± 0.727)	0.000*		Afar (26.643± 0.727)	0.000*
	MTI (28.53 ± 0.728)	0.020*		MTI (28.53 ± 0.728)	0.000*
	Ediget (11.435±0.441)	0.000*		Ediget (11.435±0.441)	0.000*
	Woff (24.875± 0.727)	0.000*		Woff (24.875± 0.727)	0.000*
	SVS (25.814±0.866)	0.000*		SVS (25.814±0.866)	0.000*

Salt sample and its iodine content in mg/kg		P-value	Salt sample and its iodine content in mg/kg		P-value
Shega (19.57± 0.167)	Risa (37.252 ± 0.603)	0.000*	Risa (37.252± 0.603)	Shega (19.57 ± 0.167)	0.000*
	Fayadhaa (17.449±1.016)	0.088		Fayadhaa (17.449±1.016)	0.000*
	Safe (31.123 ± 0.29)	0.000*		Safe (31.123 ± 0.29)	0.000*
	Waliya (20.984±0.441)	0.505		Waliya (20.984±0.441)	0.000*
	Afar (26.643± 0.727)	0.000*		Afar (26.643± 0.727)	0.000*
	MTI (28.53 ± 0.728)	0.000*		MTI (28.53 ± 0.728)	0.000*
	Ediget (11.435±0.441)	0.000*		Ediget (11.435±0.441)	0.000*
	Woff (24.875± 0.727)	0.000*		Woff (24.875± 0.727)	0.000*
	SVS (25.814±0.866)	0.000*		SVS (25.814±0.866)	0.000*

*mean difference is significant at $P < 0.05$

As shown in **Table 7,** Risa salt showed significantly highest amount of iodine content (37.252 ± 0.603mg/kg) compared to other commercial table salts available in Debre Tabor town, this shows that there is statistically significant difference between mean iodine content of Risa compared with all other salt samples mean iodine content ($P < 0.05$). In contrary, Ediget showed significantly lowest iodine content (11.435±0.441mg/kg) compared to all commercial table salts, this indicates the presence of statistically significant difference between mean iodine content of Ediget when compared with all other salts mean iodine content. However, there is no statistically significant difference between mean iodine content of Woff, Afar and SVS ($p > 0.05$), MTI and Afar ($P > 0.05$), Waliya and Shega ($P > 0.05$), Fayadhaa and Shega ($P > 0.05$), Afar compared with Woff, MTI and SVS ($P > 0.05$). But there is statistically significant difference between

mean iodine content of Risa, Ediget and Safe compared with each of all mean iodine content of salts ($p < 0.05$).

4.3. Comparisons of level of iodine with WHO and Ethiopian standard

Table 8: Comparisons of level of iodine with WHO standard

Level of iodization according to WHO	n	Proportion (%)
Non iodized (<5 ppm)	0	0
Insufficiently iodized (5-14.9 ppm)	1	10
Adequately iodized (15-40 ppm)	9	90
Over iodized (>40 ppm)	0	0

n = number of salt sample

As seen in **Table 8**, according to WHO recommended level of iodization, the iodine content of salt samples from the total of ten (n = 10) salt samples, 10% (n = 1) was insufficiently iodized, 90% (n = 9) were adequately iodized. Of salt samples collected from the study area, none were non-iodized and over iodized. Almost all salt brands collected from Debre Tabor contained adequate amount of iodine (15-40ppm). So, according to WHO's recommended level of iodization, it can be predicted that most of the people of Debre Tabor were not deprived of required amount of iodine in the salts. In Ethiopia, iodization level 36-48 ppm is adequate level for human consumption according to QSAE (Gerensea *et al*., 2016). According to QSAE's recommended level of iodization, all different salt brands commercially available in Debre Tabor town did not contain adequate level of iodine except Risa salt, which contained adequate level of iodine out of ten salt samples collected. All of inland salt samples contained iodine content below adequate range (36-48 ppm) set in Ethiopia. This indicates absence of adequately iodized salt at Debre Tabor town. This may be due to long exposure to moisture and sunlight. Salt samples stored under light lose more iodine than the salt samples stored in shade. In one study, conducted in New Delhi there was about 31% loss of iodine from iodized salt due to exposure to sun light (Gebremariam *et al*., 2013), packing nature and storage place at production and retail level. Another study conducted in Canada showed that iodine content of salt remained constant and its distribution remained uniform for many months when the salt is packed and kept dry in

cool place and away from light (Meselech Regassa *et al*., 2016). One reason for loss of iodine content may be non-homogeneity of iodine in iodized salt during iodization or salt packaging. A group (Dasgupta *et al*., 2008) studied the homogeneity in single can of iodized salt and the researcher found that iodine content varied in samples taken from the same container of different depth (top, middle and bottom). And it may be due to weakness of quality control and assurance in controlling and monitoring iodine content in iodized salts during iodization, storage and distribution. So, the government of Ethiopia should establish different laboratories at national, regional or sub regional level and networking them for confirming iodine level in order to control and prevent IDD. In this study, according to level of specification set by QSAE, only (10%) out of ten salt samples contained adequate iodine content which is the same compared to the study conducted in Senegal (10%), but, it lower compared to study conducted in Kenya in 2015 (26.2%), in Sudan (14.4%) and in Haiti (11%) in 2012. According to WHO's recommended level of iodization, the proportion of adequate iodine content in salt in this study is (90%) which is lower compared to study conducted in Nigeria (95%) and higher compared to Ghana (75.6%), India (64%) and Tajikistan (71%). The reason for this may be the difference in technology to refine iodized salt, lack of enforcement in legislation and quality-controlled iodization technology at production site, correct labeling, storage and packaging (Anteneh *et al*., 2017). One study conducted to determine the content of iodine out of 99 salt samples collected from Palestine showed that 43.5% of samples fit to Palestinian standards institute recommendation (35-55ppm) while 58% fit to UNICEF recommendation (15-40 ppm). A study conducted for determination of iodine content in different brand of table salts in India showed that most of the salts collected contained iodine level in acceptable range (15 ppm and above) some up to 30 ppm. Out of five samples only one was shown to have poor iodine content (Ajmal *et al*., 2018) and similar study conducted in Bangladesh showed that Out of the 7 samples, only one was shown to have poor content of iodine (Fardousi, 2012), but that may be due to long exposure to moisture while working in laboratory, inaccuracy and oversight due to faulty equipment (Fardousi, 2012; Ajmal *et al*., 2018). Another study conducted in Dera district; north-west Ethiopia identified that three (60%) out of five salt samples had acceptable iodine content according to WHO's recommended level (Anteneh *et al.*, 2017) which is lower compared to this study (90%).

5. CONCLUSION

Iodine is indispensable micronutrient to human and required in trace amount for well-being of individuals, and to prevent IDD. Iodine supplementation is the most effective solution to prevent IDD in areas deprived of iodine rich food. Mostly used method to address the problem of iodine deficiency is iodization of salt with iodine (salt iodization). Salt iodization involves the addition of predetermined amount of iodine to the salt during production process to ensure the correct dosage of iodine as stipulated by government standard. It is first line public health measure for preventing and controlling IDD. The iodine content of different salt brands was determined by iodometric titration and RTK. According to WHO's recommended level of iodization, out of ten salt samples 90% of salt samples contained adequate level of iodine. But according to QSAE or iodization level set in Ethiopia, only Risa salt had adequate iodine level. So, 90% of salts commercially available in Debre Tabor town for human consumption did not contain adequate amount of iodine. Out of ten salt samples Risa showed significantly highest amount of iodine content compared to other commercial table salts available in Debre Tabor town. In contrary, Ediget showed significantly lowest iodine content compared to all commercial table salts.

6. RECOMMENDATION

According to recommended level of iodization set by QSAE, almost all of salt samples available in Debre Tabor town did not contained adequate amount of iodine; so, the following are recommended;

- ✓ Establishing different laboratories at national, regional or sub regional level and networking them in order to control and prevent IDD.
- ✓ USI program in Ethiopia needs strict quality assurance measures at the stage of production, retail and consumption level for successful and sustainable prevention of IDD.
- ✓ Other iodine supplements should be added along with the diet to prevent IDD.

7. STRENGTH AND LIMITATION OF THE STUDY

7.1. Strength of the study

Determination of iodine content in salt samples commercially available in Debre Tabor town was attempted for the first time in Debre Tabor. Therefore, this study will provide baseline information for concerned bodies involved in determination of iodine content in salt samples commercially available in Debre Tabor and other area of the country for further study.

7.2. Limitation of the study

This study determined the content of iodine at shop level only. However, salt may lose its iodine content at production level or transportation before arriving retail level.

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