**An-Najah National University**

**Faculty of Graduate Studies**

# Determination of Iodine Level in Consumer Table Salt from Production to Consumption in Palestine

**By**

**Randa Taleb Rajabi**

**Supervisor**

**Dr. Maather Sawalha**

**Co. supervisor**

**Prof. Ansam Sawalha**

**This Thesis is submitted in Partial Fulfillment of the Requirements for the Degree of Master of Environmental Science, Faculty of Graduate Studies, An-Najah National University, Nablus- Palestine.**

**2016**

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**This Thesis was Defended Successfully 19 / 9 / 2016 and Approved by:**

| **Defense Committee Members** | **Signature** |
| --- | --- |
| – Dr. Maather Sawalha / Supervisor | ………………… |
| – Prof. Ansam Sawalha / Co. Supervisor | ………………… |
| – Dr. Raed Alkwoni / Internal Examiner | ………………… |
| – Prof. Radi Dawood / External Examiner) | ………………… |

# Dedication

I wish to dedicate this thesis to the most important people in my life "My Parents" who always have encouraged me to pursue science, progress, acquire knowledge, and gave me hope and strength to complete this work.

To my sisters who helped and supported me, and to my brothers who have always encouraged me.

# Acknowledgment

First, I would like to express my gratitude to ALLAH who gave me the help and power to complete this work until the end.

I would to sincerely thanks and gratitude to my supervisors, Dr. Maather Sawalha for her continuous support, guidance and encouragement, and Prof. Ansam Sawalha co. supervisor for her guidance, reading, and help in the thesis.

Thanks also to the technical staff at Chemistry Department at An-Najah National University during the experimental and analysis work.

Thanks for my friends in Al Fatat Al Laji’a “A” school in Jerusalem for encouragement.

Great thanks for my best friend Zainab Bukhary for her help and support.

Thanks to all people who supported me to end this research.

## الإقرار

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

**Determination of Iodine Level in Consumer Table Salt from Production to Consumption in Palestine**

أُقر ان ما شملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، استثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل أو جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي لدى أي مؤسسة تعليمية أو بحثية أخرى

## Declaration

The work provided in this thesis unless otherwise referenced is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

**Student Name:** رنده طالب ارجبي **اسم الطالبة:**

**Signature:** رنده **التوقيع:**

**Date** ١٩/٩/٢٠١٦ **التاريخ:**

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## Abstract

Iodized salt is the best ways known currently eliminate iodine deficiency disorder (IDD) and thyroid diseases. Iodate and Iodide are the two forms in which iodine is added to table salt. The concentration of iodine in iodized salt is affected by light, heat, time and other parameters. In Palestine, there are no studies shows the concentration of iodine in commercial packages. To determine the concentration, 99 samples of salt were randomly collected from Palestinian consumers in different governorate, it was found that 23-28% of samples have lost iodine content by 61-80% and 9% have lost up to100%. And about 43.5% of samples fit to Palestinian Standards Institute recommendation (35-55mg/kg) while 58 % were fit to UNICEF recommendation (15 mg/kg), and there is no noticeable difference in iodine concentration in packages during consumption. Also the study showed that the iodine lost during wet heating method was up to 67.5%, while in the dry heat was up to 26% only, and the iodine was lost in light more than in dark place.

Nine out of twelve iodized salt brands were collected from the Palestinian market, and results show that most of them are almost homogenous and the standard deviation between top, middle and bottom of the package ranged from $1.25 \times 10^{-4}$ to $2.17 \times 10^{-2}$. However, more attention should be paid to this subject by consumers, decision makers and researchers.

# Chapter One

# Introduction

## 1.1. Iodine

### 1.1.1. Physical and chemical properties

The French scientist, Bernard Courtois, was the first who discovered iodine in 1811. Iodine is an element (symbol: I) has a 126.9045g/mol atomic weight and atomic number of 53. Its electronic configuration [Kr] $5s^2 4d^{10} 5p^5$. The chemical element suggests the valence of +1, +3, +5which are possible. Iodine is found as $I^{-127}$ in the environment, which is considered the stable form, and it is found as $I^{-129}$and $I^{-131}$ formulas, which is the radioactive form of iodine. Iodine forms a blue-violet vapor at room temperature with 11.2 g/L gas density in 1 atm (Liu, 2007). The inhalation of the vapor causes irritating to the eyes, nose, and throat. It is soluble in most organic and inorganic solvents where it is slightly soluble in water, which the solubility of water can be increased as sodium or potassium iodide (Kaiho, 2014).

### 1.1.2. Iodine sources

According to Patrick (2008), the oceans are the worldwide repository of iodine; very little of the earth's iodine can actually be found in the soil. In order to produce the Iodine, it should be washed away from terrestrial crust to the sea, and causes the iodine scarcity in the earth’s surface which it is rich in iodine (Baker *et al*., 2000). Iodide has uneven distribution in the earth, it is found in either inorganic or organic form in water (seawater or oceans) and in soil. Iodine comes from iodide ions in seawater, which are oxidized then volatilized and evaporated into the atmosphere, returned to the soil by rain (Zimmermann *et al.*, 2008). Diverse food contains iodine, most foods provide 3 to 75-µg iodine per serving, but this can be affected by the content of soil in which it was planted, irrigation and fertilizers. Marine foods have higher concentration of the element when compared to other food since marine animals concentrate iodine from seawater (Zimmermann, 2012). Iodine forms important content of food groups: fish, marine and shellfish, eggs, dairy products (yoghurt and quark milk and cheese), vegetable and fruits (apples, avocado, banana, tomato, eggplant and nuts like cashew). Cereals (brown and white bread, corn bread, biscuit and cake) also have the element (Haldimann *et al.*, 2005).

### 1.1.3. The importance of iodine for the human body

The body cannot store iodine for long periods and therefore a daily supply is required. Iodine occurs in a variety of chemical forms, such as iodide ($I^-$), iodate ($IO_3^-$) and elemental iodine ($I_2$). In human nutrition, iodine is generally absorbed as iodide (Grewal, 2011). Iodine is an essential trace element required for the synthesis of the thyroid hormones by the thyroid gland: tetraiodothyronine ($T_4$) and triiodothyronine ($T_3$). Both formulas are essential for mammalian life, human metabolism,. They are important for growth and organ development (brain, muscle, heart, pituitary and kidney) (Khazan *at el*., 2013).

Thyroid hormones which depend on iodine regulate many key biochemical reactions in the human body, especially protein synthesis and enzymatic activity (IOM, 2001). The ($T_4$) is the hormone which has the highest concentration and it is converted into ($T_3$) hormone in the cytoplasm. ($T_3$) is the active form of thyroid hormone (Bianco and Kim, 2006).

## 1.2. Thyroid gland

### 1.2.1. Description

The thyroid gland is an endocrine gland; it has a butterfly shape and it is located in front of the windpipe, below the voice box. Thyroid has two side scaled lobes connected in the middle with an isthmus (Troncone *et al.*, 1993). The thyroid gland uses iodine from food to make its hormones. Thyroid gland stores these hormones and releases them according to the need (Bianco and Kim, 2006).

### 1.2.2. Function

The pituitary (a gland in the brain) secretes thyrotrophic or thyroid-stimulating hormone (TSH) which is responsible for the regulation of the thyroid functions. When the level of thyroid hormones decreases in the circulation, the pituitary gland secrets TSH hormone which stimulates the thyroid gland to produce more hormone (Denise, 2000). TSH affects several sites within the thyrocyte (the thyroid epithelial cells), the principal actions being to increase thyroidal uptake of iodine and to break down thyroglobulin (protein produced by the follicular cells) of the thyroid in order to release thyroid hormone into the circulation (Grewal, 2011). If the thyroid gland secretes excessive amount of T3 and T4 hormones, the situation is called hyperthyroidism. In this case, the metabolic rate increases, the patient will have symptoms of nervousness, rapid heartbeat, rapid growth of hair and nails, hyperthermia, exophthalmus, and other symptoms may occur due to hyper functioning of thyroid gland. On other hand, hypothyroidism occurs due to a decrease in thyroid hormones secreted, thus the metabolic rate slows, and the patient becomes cold intolerant, has rough dry skin, suffers hair loss and other symptoms (levy, 1991).

Hypothyroidism increases as the person becomes older and affectes women more than men (5:1). Iodine deficiency causes hypothyroidism, since the insufficient iodine intake leads to the thyroid hormones production (Koumourou, 2004).

### 1.2.3. Iodine absorption by the Thyroid gland

The iodine that comes from foods and water is converted into the iodide ion before it is absorbed by the gastrointestinal tract. The Iodine enters the circulation as plasma inorganic iodide, then it is removed from circulation by the thyroid gland and kidney. $T_3$ and $T_4$ hormones are synthesized from iodination of the amino acid tyrosine through several steps. The steps are:

(1) The transfer of iodine from circulation into the follicular cells.

(2) Synthesis of thyroglobulin.

(3) Oxidation of iodide into iodine.

(4) Iodination of tyrosine.

(5) Coupling (two molecules of diiodotyrosine).

(6) Storage in colloid in follicles.

(7) Secretion into circulation (Griffin and Ojeda, 2004).

The iodide is used by the thyroid gland for synthesis of thyroid hormones, and the kidney excretes iodine in the urine. The excretion of iodine in the urine is a good measure of iodine intake (Vejbjerg *et al*., 2002). Thus the thyroid gland concentrates iodide in amounts required for adequate thyroid hormone synthesis, and most of the remaining iodine is excreted in urine. Urine contains more than 90 percent of all ingested iodine (Koumourou, 2004).

### 1.2.4. Recommended dietary intake

Iodine is a mineral essential for healthy growth of the human body, and the body cannot synthesize it. The daily dietary iodine intake is varied throughout the world; it depends on the iodine content of soil and water in addition to culturally established dietary preferences (Stewart and Wass, 2011).

The world health organization (WHO) recommends a daily intake of 90µg of iodine for preschool children (up to 59 months), 120µg for schoolchildren (6 to 12years), 150µg for adolescents and adults, and 250µg for pregnant and lactating women (WHO, 2007).

## 1.3. Iodine Deficiency Disorders (IDD)

### 1.3.1. Definition

Iodine deficiency occurs in situations of inadequate iodine intake. This can affect thyroid hormones and consequently may cause many problems for the human body (Zimmermann, 2012). This diverse array of iodine deficiency problems in different age groups is described as Iodine Deficiency Disorder (IDD). About 15-20 mg of iodine is found in the healthy body of adults, a 70-80% is stored in the thyroid gland (Ahad and Ganie, 2010). In this case of iodine content which has less than 20 µg, it is classified as chronic iodine deficiency (Zimmermann *et al*., 2008).

### 1.3.2. Causes

The main cause of IDD is the lack of availability of iodine from the soil linked to a severe limitation of food exchanges (Thilly *et al*., 1992). So IDD happens when the amount of iodine in food (Iodine deficiency) is lower than the body’s need, consequently; the thyroid gland will not be able to synthesize a sufficient amount of thyroid hormones (Kapil, 2007). In addition to iodine deficiency, several minerals and trace elements are essential for normal thyroid hormone metabolism, e.g., iron, selenium, and zinc. Deficiencies of these elements can impair thyroid function. Iron deficiency for example, impairs thyroid hormone synthesis by reducing activity of heme dependent thyroid peroxidase (Zimmermann and Köhrle, 2004). Also it is found that the environmental toxicants, pollutants and goitrogenic factors such as: polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), organochlorine pesticides (DDT, TCDD), group includes CNS-acting drugs (phenobarbital, benzodiazepines), phenol derivatives and others were effective on the syntheses of thyroid hormones (Preace and Braverman, 2009; Davis, 2009) Other factors including some of food examples: sweet potato, soy, broccoli, turnip, cauliflower and others could cause IDD (Gaitan, 1990; Zimmermann, 2012).

### 1.3.3. Deficiency of iodine in soil

Iodine deficiency happens when there is lack of iodine in the earth crust. Iodine does not occur naturally in specific foods, and when iodine concentration is lost from the soil by washing, it leads to loss of iodine absorption in the food that grows in that soil world (WHO, 2007; Kapil, 2007). Goiter and cretinism (two forms of IDD) were found to occur in high mountain ranges, rain shadow areas and central continental regions (Fuge, 2012). Thus, iodine deficient soils are common in inland regions, mountainous areas and places with frequent flooding; also, it can also occur in coastal regions (Zimmermann *et al.*, 2008).

### 1.3.4. Symptoms of IDD

In severe iodine deficiency, insufficient amounts of thyroid hormones are synthesized. This leads to several conditions such as goiter (results from increased thyroid stimulation by thyroid stimulating hormone (TSH)), cretinism, mental retardation, decreased fertility rate, increased prenatal death, and infant mortality and others could be happen (Delange, 2009). The insufficient production of thyroid hormones also causes hypothyroidism, which leads to slowing down of metabolic processes in the human body. Several symptoms can occur: fatigue, weight gain, difficulty losing weight, slow reflexes, poor memory and concentration, muscle weakness and cramping, cold intolerant, decreased sweat, dry skin, increasing cold sensitivity, constipation and depressed mood, thin and brittle hair and nails and absent or infrequent menstrual cycle. Moreover, insufficient production of thyroid hormones can lead to decrease heart and lungs functions in addition to hypertension (Levy, 1991).

### 1.3.5. Status of IDD in the world

Despite the fact that most countries in the world are working to eliminate IDD, It is still found in different parts of the world. The percentages of IDD and iodized salt used differ from one country to another, thus number of individuals with IDD differs from region to another according to the iodine intake per day. Lifestyle and the place of residence are other factors that lead to differences between individuals with IDD (Kapil, 2007).

In 2013, 111 countries found to be having sufficient iodine intake, whereas thirty countries remain iodine deficient; 9 are moderately deficient, 21 are mildly deficient, and none are currently considered severely iodine deficient. However, ten countries have excessive iodine intake (Pearce *et al*., 2013). WHO refered that there were 110 countries have iodine deficient in 1993, (WHO, 2014). Whilst the Iodine Global Network (IGN) showed that the number of IDD countries has almost halved in the past 12 years, and the number decreased from 55 to 26 (IGN, 2016).

Fuge, ( 2012) indicated that there is about 2 billion people worldwide have IDD while the majority of iodine deficiency was concentrated in the developing world, and it was re-emerging in industrialized countries .

### 1.3.6. Prevention of IDD

The most effective solution to address the problem of IDD in areas deprived of iodine rich food is iodine supplementation. Iodine deficiency can be corrected by adding iodine to dietary media like salt, oil, water, sauces etc. (Ahad and Ganie, 2010), Campaigns for prevention of iodine deficiency have been instituted in many countries, and salt iodization has been the most successful (Cellejas, *et al.*, 2016).

A supplementation of table salt with iodine is the mostly used method to prevent IDD worldwide (Liu, 2007). The universal salt iodization (USI) can be used for all human needs plus livestock consumption. Table salt with iodine is considered the most effective way to control iodine deficiency. On the other hand, some countries use different ways for the fortification with iodine; some add iodine to bread, some iodizes drinking water, milk, animal fodder and iodized oil (Zimmermann *et al.*, 2008). The prophylaxis and therapy of IDD can be achieved extremely efficiently by intramuscular injections or oral iodine (Delange, 2009).

Iodine is added to salt in the form of potassium iodide (KI) or potassium iodate ($KIO_3$), either as a dry solid or a sprayed aqueous solution at the point of production (Liu, 2007).

## 1.4. Iodized salt

### 1.4.1 Background

Iodine was first added to salt in 1924 in the United States (Markel, 1987). In 1994, the WHO and the UNICEF recommended universal salt iodization (USI) as the main strategy to elimination IDD. Additionally WHO/UNICEF/ICCIDD recommend that the amount of added iodine should be 20-40 mg iodine per kg of salt (20-40 ppm of iodine) at the point of production, which can provide 150 μg of iodine per person per day. This is because 20% of the iodine in salt is lost during the production process for household use, and another 20% is lost during cooking process. Another 50% could be lost during packaging, storage, and heat (WHO 2007).

### 1.4.2. Stability of potassium iodate

A study carried out by the WHO (2007) showed that when iodine was given as iodate, it is available to the thyroid gland and it has no toxic effects. Additionally, the study demonstrated that potassium iodate is as effective as potassium iodide for the prevention of endemic goiter, thus potassium iodate is more recommended than potassium iodide because of its stability.

## 1.5. The Situation in Palestine regarding the thyroid and iodine

### 1.5.1. Iodine deficiency disorder in Palestine (West Bank and Gaza)

A survey documents the prevalence of goitre in 1997 that included 2535 schoolchildren, found that the total goiter prevalence was 14.9% in the age group of 8-10 years distributed among West Bank and Gaza (1535and 1000 respectively). The lowest rate of goiter was in Qalqelia governate (0 %) and the highest was in Jericho governate (68.2%). By region, it was found to be lowest in the northern (3.9%) region of the West Bank as well as Gaza Strip (5.6%) and highest in the middle (31.6%) and southern (31.9%) regions of the West Bank. The distribution of median iodine excretion in the urine ranged between 2.74μg/dl in Jericho, which was the lowest; to 18.59 μ g/dl. On the other hand, the highest was in Salfit has. Girls were slightly more likely to have goitre than boys (Ramlawi and Abdeen , 1997).

The last report of PMOH (2013) showed that 1200 samples were collected from West Bank and Gaza strip (600 from WB & 600 from Gaza), from 88 primary schools and clinics and analysed by Photometric detection. The results revealed that median iodine extraction was higher in children living in Gaza compared to those in West Bank (239 and 153µg/L; respectively), The median for Palestine was 193µg/L, and the percentage of IDD was 33.6% for student in West Bank and 17.3% for those in Gaza strip (PMOH, 2014) .

### 1.5.2 Consumption of iodized table salt

The Palestinian government and the Palestinian Ministry of Health (PMOH) are working to eliminate IDD in the West Bank and Gaza by iodizing table salt, which is used by almost every household in cooking (PMOH, 2014).

In 2004, a survey entitled *Demographic and Health Survey (DHS)*, conducted on 5779 households, showed that there was 65.3% of the household in Palestine consuming iodized salt (15 ppm and more) distributed as 56.5% in West Bank and in 82.7% Gaza (PCBS, 2004).

In the Palestinian family survey report 2010, the salt test kit was used to study the consumption of iodized salt among 12158 households in the different regions of West Bank and Gaza. The results showed that 77% of households in Palestine used greater than 15 ppm[1] of iodized salt,

[1] The indicator used in the survey ( more than 15 part per million iodine in salt)

distributed as 91% in Gaza strip and 68% in West Bank strip (PCBS, 2013).

The report published by the PCBS in 2014 entitled "*A multiple indicator cluster survey* - *MICS*" showed the consumption of iodized salt (15 ppm or more) was happening in 74.5% households in Palestine (69.3% in West Bank and 79.7% in Gaza) (PCBS, 2015).

In the report entitled "*Palestinian micronutrient survey (PMS) 2013*" conducted by the PMOH, the results also have been adopted in its final annual report for 2013. The report showed that a titration method was used to test the finding of iodine in table salt for 1200 Palestinian households distributed as 50:50 between West Bank and Gaza. The report depended on the Palestinian Technical Regulation and UNICEF recommendation to study the percentage of iodine content on table salt used by tested households. (Table 1) shows the percentage of salt samples in Palestine according to the quality of iodized salt based on Palestinian Technical Regulation. Table (2) shows the percentage of iodized salt samples in Palestine (greater and lower) than UNICEF recommendation, which is 15 mg/kg. (PMOH, 2014).

**Table 1: The percentage of iodized table salt samples in Palestine based on the Palestinian Technical Regulation recommendation**

| Quality of salt iodization | Palestine % | West Bank% | Gaza strip% |
|---|---|---|---|
| Not iodized | 9.5 | 18.8 | 0.3 |
| Very low <(25mg/kg) | 39 | 27.2 | 50.8 |
| Low (25-34.99 mg/kg) | 45.4 | 44.7 | 45.9 |
| A adequate(35-55 mg/kg) | 5.9 | 9.1 | 2.7 |
| Excessive > (55mg/kg) | 0.3 | 0.2 | 0.3 |

The result according to the PMOH 2014 report.

**Table 2: The percentage of iodized table salt samples in Palestine based on the UNICEF recommendation:**

| Area | <15mg/kg | ≥15 mg/kg |
|---|---|---|
| West Bank | 31.1 | 68.9 |
| Gaza strip | 24.9 | 75.1 |
| Palestine | 28 | 72 |

The result according to the PMOH 2014 report.

The Palestinian micronutrient survey (PMS) report also classified salt iodization into categories of non-adequate, adequate and excessive 93.9%, 5.9%and 0.3, respectively (PMOH, 2014).

All reports discussed before showed that the percentages of iodized salt in Gaza strip are more than in West Bank. The reports also showed that using iodized salt in the different areas in Palestine was different from one area to another; however these differences were not significant.

### 1.5.3 Source of iodized salt in Palestine

There is a single Palestinian factory produces salt in Palestine called "The West Bank salt Company" located in a restricted military area along the shores of the Dead Sea. The factory was licensed by the Jordanian government before 1967 and it was later licensed by the Israeli Ministry of Industry and Trade. The factory is accredited by the Palestinian Standards Institute (PSI) and the International Federation of Organic Agriculture Movements (IFOAM). The factory adds iodine to salt in the form of Potassium Iodate ($KIO_3$), which is imported from France. $KIO_3$ is sprayed by a pump when salt passes in the production stages. The produced salt is packaged in paper or plastic bags with a capacity of 1, 25 and 50 kilogram (PMOH, 2008).

The factory produces about 11000 tons of salt per year. Because of the competition with the Israeli factories, the West Bank salt company has been facing difficulties in the marketing operations, particularly, in the domestic market (PMOH, 2008).

### 1.5.4 The salt packaging factories

Salt packaging factories are distributed between Nablus and Hebron. The packaged salt is iodized according to the factories owners, such factories include: Al shelleh, Al Karaky Company, Mody Packaging Market, Maswady for Trade and Food Stuffs and Al Motahedon Trade Company. And their mission depends on the packaging of the salt that has been bought from producing factories. All the packaged salt is sold in the local market, which comes in packages of 1kg plastic or paper bags, and only Maswady Company has a package of 25 kg salt in plastic bag (PMOH, 2008).

From the same study, about 82.1% out of 56 types of table salt trademarks sold in the Palestinian market are Israeli salt, only15% are Palestinians, and the remaining was an imported salt.

More than 87.3% of salt bags don’t have a food fortification sign on the bags, 36.1% of Palestinian salt are not matching with the Palestinian specifications of the packaged salt (PMOH, 2008).

## 1.6. Objectives

### 1.6.1. General Objectives

The main objective of this project is to determine iodine level in table salt in consumer salt in Palestine from production stage until consumption by using spectrophotometry method.

### 1.6.2. Specific objective

The project aims to achieve the following objectives:

1. To study the effects of selected environmental variables on iodine levels in table salt (temperature, time , heat upon cooking ).
2. To compare the levels of iodine in consumer salt with the Palestinian and international standard.

3. To compare iodine concentration between the different salt brands collected from the local market.
4. To quantifying iodine loss from production to consumption.

# Chapter Two

# Literature view

## 2.1. Methods used to determine iodine

Several methods can be used in determining iodine in salt, urine, milk and serum. Such methods includes: titration, rapid testing kits, instrumental neutron activation analysis (INAA), electrochemical detection (ED), flow injection analysis (FIA) x-ray fluorescence, (XRF), inductively coupled plasma mass spectrometry (ICP-MS), sandell-Kolthoff reduction, radiochemical neutron activation analysis and capillary electrophoresis, The last fifth methods are the most commonly used in salt analysis (Khazan *et al*, 2013).

### 2.1.1 The titration method

Many studies expressed and agreed that the titration method is the one mostly used worldwide in the determination process of iodine quantity in the iodized salt. This is due to its accuracy, ease of operation and low cost, but it takes a lot of time to perform its task (Khazan *et al*, 2013), requires capital infrastructure and trained personnel (Pandav *et al*, 2000). The iodine content of salt is determined by liberating iodine from salt and titrating the iodine with sodium thiosulfate using starch as an external indicator. The method of liberating iodine from salt varies depending on whether salt is iodized with iodate or iodide (Khazan *et al*., 2013).

### 2.1.2. Rapid testing kits method

Rapid test kits method are suitable for qualitative use in situations where iodized salt need to be distinguished from non-iodized salt, and are frequently applied in household studies ( Khazan *et al.*,2013). Iodine spot-testing kits do not require any infrastructure, they are inexpensive, they are most importantly and they provide immediate results suitable for rapid feedback. The kits give either a qualitative or a semi-quantitative estimation of the iodine content. The test gives no indication of the actual level of iodine in the salt (Pandav*et al.*, 2000).

### 2.1.3. Inductivity coupled plasma-mass spectrometry (ICP-MS)

ICP-MS method has been used in different studies specially in quantifying iodine in different forms of food such as plants, sea water, fish, soil, milk powder, egg powder, cod muscle, pig kidney and hay powder (Karen *et al.*,1997). ICP-MS is more accurate than the titration method. In this method, iodine is detection is done, yet it is not easily applicable because it needs a high level of specialization and are expensive so it is not a practical method (Khazan *et al.*, 2013).

### 2.1.4. Spectrophotometric method

Spectrophotometry is a good quantitative method to determine iodine levels (Jooste and Strydom, 2010). It is a widely used method in determination of iodine in the form of iodide or iodate and periodate. Different studies used this method, which is based on liberating iodine from potassium iodate in acidic medium. The liberated iodine reacts then with a chromogenic reagent to form tri-iodide. Reagents used in the method include 3,4-dihydroxybenzalde-hydeguanylhydrazone,isonicotinicacidhydrazide, 2,3,5-triphenyl tetrazoliumchloride, dithizone, methylene blue , thionin, azure B, and others (Cherian *et al* , 2007; Narayana *et al*, 2006).

In this study, spectrophotometric method is used in determining iodine in salt by using a methylene blue as a reagent. Methylene blue is used for the determination of iodate in salt and seawater samples (Narayana *et al*, 2006)

Methylene blue (MB) is a dark green, odorless crystals with bronze luster or crystal powder. The molecular weight 319.85 g/mol and Molecular Formula is $C_{16}H_{18}ClN_3S$, it's chemical name is [3.7-Bis(dimethylamino) phenothiazine-5-ium chloride].

Methylene blue is prepared from dimethyl aniline and thiosulfuric acid. It is soluble in water and alcohol, but insoluble in ether. It is used as a reagent for several chemicals, oxidation-reduction indicator, and stain bacteriology (Windholz *et al*, 1983).

## 2.2. Determination of iodine in the world

Studying iodine intake is a great interest for most countries in the world according to its Importance for human metabolism and thyroid synthesis, and this included studying iodine intake in table salt and many foodstuff in addition to the effects that may could causes loss of iodine intake. Whereas some studies goes to show urinary iodine concentration (UIC), or serum thyroid hormone concentrations.

In a study created in 2009 in New Zealand, to determine the level of iodine in retail salt samples, the study showed that a 20 different salt products were studied by taking five different batches of each product. The study used inductively coupled plasma-mass spectrometry (ICP-MS) for the analysis. The mean concentration of iodine in iodized table salts ranged from 32-64 milligrams of iodine per kilogram of salt (mg/kg), within the requirements of Australia New Zealand Food Standards 25-65 milligrams of iodine per kilogram of salt (mg/kg) (Thomson, 2009).

By other study, the iodometric titration method was used in determination of iodine concentration in different salt brands collected from different areas of Bangladesh. The study showed that only one out of seven brands has a poor content of iodine (Fardousi, 2012).

Liu, (2007) studied the content of iodine and iodate in different table salt brands, where potassium iodide (KI) was used in US, so the iodine content of the salt samples was measured by ICP-MS with Ge as an internal standard. Whereas in Asian countries (where Chilean Caliche ,India, China, Thailand and Australia chosen in the study), which they used the iodate in salt in an iodized salt program an electrochemical detection system in which aqueous iodate is reduced on a stainless steel working electrode with a platinum auxiliary electrode in acidic medium. Under optimized applied voltage, the electrochemical reduction current is directly related to iodate concentration in the samples. The study confirmed that the loss of iodine from iodized salt under humid conditions and high temperature was due to that it might oxidizes and sublime. Also based on the analysis of many samples from providers across the US, a large fraction of salt samples do not contain the amount of iodine stated on the labels (Liu, 2007).

Another study which used an iodometric titration method to analyzing iodine content in a salt samples that were collected from the 12 producers in South Africa. Three Samples were taken from each producer (500 g-1kg) freshly produced, and samples from batches of iodized salt produced before two days, and samples from the repackage sites. Result showed that the legal requirement of 40–60 ppm iodine was met in 30.9% of salt samples; 57.9% contained more than 30 ppm iodine; 34.8% contained under 20 ppm iodine. The study also showed that there were shortcomings in perceptions and knowledge about iodine deficiency disorders and in the internal quality control procedures of a substantial proportion of the producers (Jooste, 2003).

Whereas in Belgrade, (Rajkovic, 2009), the contents of KI in samples of table salt bought in Belgrade supermarkets “seven manufactory”, was studied by using a volumetric method of indirect iodometry. The results of all received table salt samples showed that they were according to the demands of Regulations (16-24 mg/kg), except one sample which contains significantly less mass of KI than what determined by Regulation.

Another study in Bahir Dar; North Western Ethiopia, which both iodometric titration and spectrophotometric method were used for the determination of iodine in salt samples commercially available (Eight fine and five coarse crystal salt samples). The results showed of all the salt samples collected from Bahir Dar found that, 61.54 % were insufficiently iodated, 23.08 % were adequately iodated and 15.38 % were over iodated. It also showed that the quality of iodized salt in Bahir Dar city is not uniform and the universal salt iodization program in Ethiopia needs strict quality assurance measures at the stages of production, storage, import and retail levels for a successful and sustainable prevention of IDD. (Bediye and Berihe, 2015).

# Chapter Three
# Methodology

## 3.1. Samples collected

Sample were solicited from households, and 99 household was responded, the consumers sample was considered complete if the consumer provided us with three subsamples from the salt package he/she is using. The first sample (later is called stage (1)) is to be provided when the consumer starts to use a package immediate when the package is opened, and the second sample (later is called stage (2)) provided when the package is half consumed, and the final sample is provided when the package is almost empty (also is called stage (3)).

A questionnaire was designed for this study, and each consumer was asked to fill it. Parameters in the questionnaire included: governorate, salt brand, salt container material used by consumer, place of used container if it was near or far the heat and light, the case of salt container after used (opened or closed), the salt consumption in a month, and other parameters (see appendix A).

About 10 gram of salt was taken from the top of the fresh container, transferred to be a thick-walled zip-lock bag, excluding air as much as possible when sealing the bag, Consumers were asked to wrap the bag with aluminum foil, and keep the samples in dark and cool area in refrigerator until being handed to the researcher with the questionnaire information.

Moreover, the samples collected by the researcher are also kept in dark area and cooled in refrigerator until analysis time.

### 3.2. Studied parameter

Samples were classified according to the producer, amount of iodate added by producer and governorate. Then the samples were studied for the following:

1) The iodine levels in salt: during consumption of salt, packages were quantified in the three sample stages and compared to the amount expected in order to be found based on what is written on the package.

2) Effect of temperature: the effect of temperature was studied to examine the effect of cooking on iodate levels; this was done by studying wet heat in boiling water and dry heat in an oven. The effect of boiling on iodate concentration was investigated by taking 5 or 10g from different brands of salt, dissolved in 250 ml of water and boiled for 20 or 30 minute. The iodine concentrations in these samples were determined and compared to their original expected concentrations. Iodate concentration was compared to the measured concentration before boiling.

For dry heat effect, 2.9215g from two different salt brand were used for the test (Shilleh salt and Salit salt), they were placed separately in an oven at both 50 °C and 100°C for one hour. Then, the salt was dissolved in distilled water and diluted up to 25 ml volumetric flask then 5 ml was taken and added all other reagents as the procedure. Then the concentration of iodine was determined and compared to their original and expected concentrations and the loss and percentage of loss were calculated. The concentration of iodine was taken for each one in the beginning before heat.

3) Effect of light: in order to study the effect of light, samples of salt were put in two small closed plastic bottles and two small closed glass bottles for about 30 days. In addition, salt sample were placed in one plastic and one glass at room light, and two others were stored in dark (by covering the bottles with silver paper). The iodine concentration in these samples was determined and compared to the original concentrations of each salt type.

4) Homogeneity of iodine distributed in salt package: Three samples of salt were taken from the top, middle, and bottom for each salt packet, the iodine concentration in each was measured then compared to concentration of others samples from the same bag.

## 3.3. Preparation of solutions

Methylene blue (MB) (0.01%) and potassium iodide (2%) solutions were prepared by dissolving 0.01g of M.B and 2 g of KI separately in distilled water, which diluted each one up to 100ml in volumetric flask. Additionally, about 1.222 g of $KIO_3$ were dissolved in distilled water, which diluted it up to 1000 ml in a volumetric flask to form 1000 ppm $KIO_3$ solution. A 2M of HCl was made by diluting a 17.5 ml of concentrated HCl in 100 ml distilled water. To make a 1M of acetate buffer solution, 0.3910 g of sodium acetate and 0.96 ml of acetic acid were dissolved in 100ml distilled water. Sodium thiosulphate solution was prepared by weighting 6 g of $Na_2S_2O_3$ and 0.1 g of $Na_2CO_3$, then dissolving it in water in 250ml distilled water in volumetric flask. Three gram of starch were dissolved in 100 ml of distilled water in order to prepare the starch solution, and 5 ml of concentrated of $H_2SO_4$ was diluted in 20 ml distilled water to prepare the diluted solution.

## 3.4. Procedure for iodate quantification

### 3.4.1. Standardizing the iodine solution

A 50 ml buret was filled with sodium thiosulphate solution and 25ml aliquots of potassium iodate solution were transferred to an Erlenmeyer flask. Then 2g of potassium iodide were added, followed by the addition of 5ml of diluted sulphric acid with rapid mixing. Then titrate the solution until a pale yellow color appears. After that, 2-3 ml of starch solution were added and titration process continues until the color disappeared (Jeffery *et al.*, 1978).

### 3.4.2. Preparation of calibration curve

An aliquot of standardized solution of iodate (0.00601 M) containing 0.25-5 ml of iodate was transferred into a series of 25 ml calibrated flasks, and 1 ml of KI 2% and 1ml of HCl (2M) were added. The mixture was gently shaken until the appearance of a yellow color, after that, a 0.5 ml of MB (0.01%) was added followed by 2 ml of sodium acetate (1M).The reaction mixture was then shaken for 2 min. Finally it was diluted with a distilled water to the mark of 25ml volumetric flask (Narayana *et al*., 2006). A blank solution was also prepared by adding all solutions as in the standards except for the iodate solution.

### 3.4.3. Determination of iodine in table salt samples

The samples were treated according to Narayana *et al*., (2006) after modification. The method suggested by the previous reference (Narayana *et al*., 2006) to treat the sample is as the following:

A total of 2.9215g table salt sample was weighted, dissolved in water and diluted up to 25 ml. A 0.5 ml of salt solution was then transferred into another 25ml volumetric flask. Thereafter, all the reagents were added as follows: 1ml KI, 1ml HCl, 0.5ml M.B and 2ml acetate buffer solutions (pH=4), respectively.

The solution was mixed well for 2 min and diluted with distilled water to 25 ml. Finally, the absorbance was measured immediately. This method was modified by using 3 ml of MB instead to 0.5 ml and 5ml from salt solution instead to 0.5 ml.

The modification occurred because the calibration curves were not linear, and $R^2$ result less than 0.90 by using the Narayana *et al.* method.

### 3.4.4. Quantification of iodate in samples

Absorbance of iodate concentration in the sample solution was measured by using spectrophotometer at 665.6 nm wavelength (Narayana *et al.*, 2006). The blank did have the highest absorbance and the darkest blue color, the degree of blue colour is proportional with the iodate content. The iodate concentration was obtained by subtracting the absorbance of the sample solution from that of the blank solution.

Absorbance loss due to iodate concentration in sample =

Absorbance of blank - Absorbance of solution

The iodate concentration was calculated by substitution the absorbance loss due to iodate in the calibration curve equation.

Methylene blue reacts with iodine in acidic medium, causing changes into blue colour, the bleaching of the blue color depends on the concentration of iodine liberated as shown in (Figure 1).

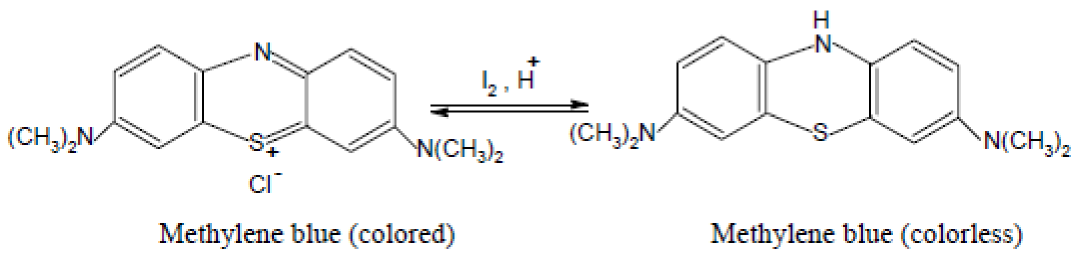


**Figure 1:** The de-colorization reaction of Methylene blue

The liberated iodine is formed by the reaction of iodate with iodide in an acidic medium. The yellow color indicates the liberation of iodine according to the following reaction:

$$KIO_3 + 5\ KI + 6HCl \longrightarrow 3\ I_2 + 3\ H_2O + 6\ KCl$$

The concentration of iodine reacted with M.B can be determined by measuring of the absorption of the solution at a wave length of 665.6nm using a spectrophotometer.

## 3.5. Instruments

Instruments were used in this study:

1. UV-visible spectrophotometry (UV-1601) with 1cm cell, Shimadzu Corporation made in Japan.
2. JENWAY (pH Meter 3510).
3. Electronic Muffle Furnace (J Lab Tech, LDO- 060E).
4. Electronic Balance (Sartorius,BP 301S).
5. Hotplate stirrer (JLab Tech, Daihan lab tech co. LTD).

## 3.6. Chemicals and reagent

The chemicals and reagents that were used in this study:

- Methylene Blue ($C_{16}H_{18}ClN_3S$) 0.01% and sodium carbonate ($Na_2CO_3$) from Sigma.
- Potassium iodate ($KIO_3$) 1000ppm and concentrated hydrochloric acid (HCl) 2M from Merck.
- Sodium thiosulphate solution ($Na_2S_2O_3.5H_2O$) from Frutarom.
- Sulfuric acid ($H_2SO_4$) from Biolab.
- Potassium iodide (KI) 2% from Chem. samuel.
- Sodium acetate ($CH_3COONa$)1M from Riedel.
- Starch.

# Chapter Four

# Result and discussion

## 4.1. Sample characterization

Samples were selected randomly from consumers in Palestinian governorate in the West Bank, where the samples created to be greater than 30 as statistical principles and studies. And total of 99 samples were collected. Most of consumers used a 1kg packet of salt from the local salt companies, which are in the region. Table 3 shows the number of samples and the different stages for every sample. Consumers provided three samples, which were taken from the begging, middle, and end of the container. The distribution of samples according to governorate is shown in table 4.

**Table3: Total salt samples and description**

| Descriptions of samples | Number of samples | Weight of salt package used by the volunteer |
|---|---|---|
| **All stages** | **64** | **1 Kg salt packet** |
| **Only one or two stages** | **30** | **1 Kg salt packet** |
| **Only one, two or three stages** | **5** | **25Kg salt** |
| **Total** | **99** | |

**Table 4: Distribution of samples according to governorate**

| | Governorate | Number of the samples |
|---|---|---|
| 1 | Jerusalem | 27 |
| 2 | Ramallah | 19 |
| 3 | Nablus | 9 |
| 4 | Hebron | 7 |
| 5 | Tubas | 13 |
| .6 | Salfit | 5 |
| 7 | Bethlehem | 3 |
| 8 | Jenin | 5 |
| 9 | Jericho | 7 |
| 10 | Qalqiliya | 1 |
| 11 | Tulkarem | 3 |
| Total | | 99 |

The collected salt samples were also classified based on the potassium iodate concentration written on the salt package (Appendix A). Most of the collected samples had 5mg/100g, 3mg/100g and 50mg/100g, as seen in Table 5.

**Table 5: Classification of salt samples according to concentration**

| Conc. on package | Number of samples |
|---|---|
| 20-40 mg/kg | 1 |
| not written | 6 |
| 50 mg /100g | 12 |
| 3mg/100g | 37 |
| 5mg/100g | 43 |
| Total | 99 |

Samples were classified into Palestinian, Israeli and imported ones, 48 samples out of 99 were Palestinian products, while 49 were Israeli, and the rest were imported.

# 4.2. Iodine levels in salt samples

## 4.2.1. Iodine loss during sampling stages

Measuring iodine concentration for all salt samples, revealed differences in concentration based on the stage (Table 6), it was divided for each 20% groups as the total result seen. The results show that 23.1-27.5% of samples lost from 61 to 80% of iodine, and about 9% of samples lost more than 80% its original iodine content that was in the package.

**Table 6: Percentage loss of iodine in the three stages for all the samples**

| % loss | % samples stage 1 | %samples stage 2 | % samples stage 3 |
|---|---|---|---|
| 1 - 20 | 28.8 | 30.0 | 33.8 |
| 21 - 40 | 27.5 | 28.6 | 24.6 |
| 41-60 | 7.5 | 10.0 | 9.2 |
| 61-80 | 27.5 | 22.9 | 23.1 |
| 80 to100 | 8.8 | 8.6 | 9.2 |
| # of samples | 80 | 70 | 65 |

The average amount lost of iodine from each sample correlated with the amount of iodine was dependent on the original amount of iodine present in each package. Based on amount added, salt can be classified into three classes (3 mg/100g, 50 mg/100g, 5 mg/100g).

The total number of the samples in each class was found, and the number of the samples in each stage in this class was found. The first class contain 3mg iodine /100g salt (0.03 mg /g), the average loss in all stages was from 0.0143 to 0.0154 mg/g in general and the min and the max are from 0.0003 to 0.03 mg/g, the result is presented in Table 7.

**Table 7: Iodine loss in the samples that were supposed to contain 3mg/100g**

| Sample stage | Number of samples[1] | Total average loss in mg/g salt package | Min. loss of iodine in mg/g | Max. loss of iodine in mg/g | Mean[2] mg/g | Remain ing of iodine mg/kg[3] | % loss |
|---|---|---|---|---|---|---|---|
| stage1 | 35 | 0.0153 | 0.0003 | 0.03 | 0.0183 | 14.7 | 51.0 |
| stage2 | 31 | 0.0154 | 0.0011 | 0.03 | 0.0188 | 14.6 | 51.3 |
| stage3 | 29 | 0.0143 | 0.0014 | 0.03 | 0.0134 | 15.7 | 47.8 |
| n=37 | | | | | | | |

1 Number of the sample from 37-consumer send salt has 3mg/100g.

2 Mean is the median loss of the samples in each stage.

3 Remaining was calculated in mg/kg to make it compared with UNICEF and Palestinian Technical Regulation.

Results in Table 7 show that the remaining iodine is less than the recommended concentration by the Palestinian Technical Regulation (35-55mg/kg), but it is almost adequate according to the UNICEF (15mg/kg) regulations.

Table 8 pertains to the samples that were expected to contain 5mg iodine/100 g salt (0.05 mg/g). The average loss in all samples was from 0.0146 to 0.0171 mg/g; their remaining amount of iodine complied with the UNICEF recommendation and is almost compliant with the Palestinian Technical Regulation. However, the median and values of maximum and minimum amount show scattering of iodine concentration around the mean in the three classes of samples**.**

**Table 8: Iodine loss in samples that were supposed to contain 5 mg /100g salt**

| Sample stage | Number of samples | Total average loss in mg/g package | Min. loss of iodine in mg/g | Max. loss of iodine in mg/g | Mean mg/g | Remain ing of iodine mg/kg | % loss |
|---|---|---|---|---|---|---|---|
| stage1 | 36 | 0.0171 | 0.00018 | 0.05 | 0.0141 | 32.9 | 34.2 |
| stage2 | 33 | 0.0154 | 0.00016 | 0.05 | 0.0122 | 34.6 | 30.7 |
| stage3 | 33 | 0.0146 | 0.00033 | 0.05 | 0.0109 | 35.4 | 29.2 |
| n=43 | | | | | | | |

On the other hand, the lass of sample that are expected to contain 50mg/100g have lost an average of 0.211 to 0.340 mg/g according to Table 9. Thus the remaining concentration of iodine is less than the Palestinian Technical Regulation, but adequate for the UNICEF standard.

**Table 9: Iodine loss in samples that were supposed to contain 50mg /100gsalt (all samples)**

| Sample stage | Number of samples | Total average loss in mg/g | Min. loss of iodate in mg/g | Max. loss of iodate in mg/g | Medi an mg/g | Remaini ng of iodine mg/kg | % loss |
|---|---|---|---|---|---|---|---|
| stage1 | 10 | 0.211 | 0.0017 | 0.3603 | 0.335 | 28.9 | 42.3 |
| stage2 | 7 | 0.336 | 0.2451 | 0.3605 | 0.355 | 16.4 | 67.3 |
| stage3 | 4 | 0340 | 0.3158 | 0.3605 | 0.342 | 16.0 | 68.0 |
| n=12 | | | | | | | |

### 4.2.2. Iodine loss during consumption of the whole samples

The samples that have three stages (complete sample), each stage has the same number of samples collected from the consumers.

Incomplete samples, which are supposed to contain 3mg/100g, the average loss of iodine in samples from stages 1, 2 and 3, is around 0.0145mg/g. The loss in all samples ranges between 0.0003 and 0.03 mg/g. The results Table 10 shows that there is insignificant decrease in the lost iodine during consumption of each sample. This deviation is about $1.04x10^{-2}$mg/g.

**Table10: Iodine loss in the complete samples, which are supposed to contain 3mg/100g of iodine.**

| Sample stage | Total average loss in mg/g | Min loss of iodine in mg/g | Max loss of iodine in mg/g | Mean mg/g | Average Standard Deviation (3 stages) |
|---|---|---|---|---|---|
| stage1 | 0.0146 | 0.0003 | 0.03 | 0.0157 | $1.04x10^{-2}$ |
| stage2 | 0.0145 | 0.0011 | 0.03 | 0.0163 | |
| stage3 | 0.0146 | 0.0014 | 0.03 | 0.0164 | |
| Number of complete samples =27 | | | | | |

Results in table 11 reveal that the 29 samples that were expected to contain 5 mg/100g had an average loss of iodine from 0.0153 to 0.0173mg /g and the loss in all samples ranges are from 0.00033 to 0.05 mg/g in the three stages. The standard deviation was $2.60x10^{-3}$.

**Table 11: Iodine loss in the complete samples, which are supposed to contain 5mg/100g of iodine**

| Sample stage | Total average loss in mg/g | Min. loss of iodine in mg/g | Max. loss of iodine in mg/g | Median mg/g | Average Standard deviation (3 stages) |
|---|---|---|---|---|---|
| stage1 | 0.0173 | 0.00033 | 0.05 | 0.0142 | $2.60x10^{-3}$ |
| stage2 | 0.0157 | 0.00016 | 0.05 | 0.0122 | |
| stage3 | 0.0153 | 0.00033 | 0.05 | 0.0113 | |
| Number of complete samples = 29 | | | | | |

As for the four complete samples expected to contain 50mg/100g, it was found that the average loss of the three stages is around 0.34 mg/g, and the loss in four samples ranges between 0.3156 and 0.3267mg/g. (Table 12) shows the results.

**Table12: Iodine loss in the complete samples, which are supposed to contain 50mg/100g of iodine.**

| Sample stage | Total average loss in mg/g | Min loss of iodine in mg/g | Max loss of iodine in mg/g | Median mg/g |
|---|---|---|---|---|
| stage1 | 0.3490 | 0.3267 | 0.3603 | 0.3560 |
| stage2 | 0.3505 | 0.3267 | 0.3603 | 0.3576 |
| stage3 | 0.3396 | 0.3156 | 0.3603 | 0.3413 |
| sample number = 4 | | | | |

Results of all studied samples show that 43.5% of them fit the Palestinian standards and about 58% fit the UNICEF ones.

Wang *et al.* (1999) studied the "*Effects of storage and cooking on the iodine content in iodized salt and study on monitoring iodine content in* *iodized salt"*. They found that about 48.3% of salt samples from markets were compliant with the national standards (30-50 mg/kg), whereas, 72.0% of samples from households were incompliant (20-50 mg/kg) (Wang *et al.*, 1999).

## 4.3. Effect of temperature on iodized salt

Three different experiments were done to study the effects of temperature on iodine in salt samples. Samples were dissolved in distilled water and boiled for 20 or 30 min then as the measurements of iodine in the solution was carried out as described previously.

Boiling salt in water for 20-30 min could cause 50.8 to 67.5% loss of iodine. The result was different according to different salt brands (Table 13).

**Table 13: Percent loss of iodine level by heat in wet salt**

| Salt brand | Concentra-ion on the package | Grams of salt | Time of boiling | % Loss of iodine at room temperature | % Loss of iodine after boiling | Net* % loss of iodine |
|---|---|---|---|---|---|---|
| Salit | 3mg/kg | 5 g | 20min | 3.12 | 61.7 | 58.6 |
| Shilleh | 50mg/100g | 5 g | 20 min | 3.63 | 71.1 | 67.5 |
| Al-safy | 50mg/kg | 10g | 30min | 16.70 | 67.5 | 50.8 |

* Net % loss for heat = % loss after boiling - % loss at room temperature.

Examining the effect using dry heat at 50 °C and 100°C for one hour on iodine content indicated that the percentage loss of iodine in salt during dry cooking increased as heat temperature increased. The maximum loss because of dry heat was 6.59 to 25.99% (Table 14).

**Table 14: Percentage loss of iodine level by heat on dry salt**

| Salt type | % loss of iodine | | | | |
|---|---|---|---|---|---|
| | At room Temperature | At 50°C | Net* % loss 50°C | At 100°C | Net* % loss 100°C |
| **Shilleh** | **3.63** | **15.62** | **11.99** | **29.62** | **25.99** |
| **Salit** | **3.12** | **9.71** | **6.59** | **28.67** | **25.55** |

* Net % loss = % loss at heat temperature - % loss at room temperature.

As shown previously, the percentage loss of iodine during boiling was more than that during dry heat.

In this study, 25% of the Palestinian consumers kept salt package close to heat source while 75% of them kept it far from heat source. (Appendix A).

Previous study of Tana and Raghuvanshi (2013) showed that the loss of iodine differs depending upon the type of cooking method and the time of cooking. Also, the study showed that loss of iodine during boiling, roasting, deep frying, and microwave cooking were found to be 40.23%, 10.57%, 10.40% and 27.13%, respectively (Tana and Raghuvanshi, 2013).

When compared with our results, since it found that all types of salt lost are more than 50% of iodine through boiling, it is advisable to add salt at the last few minutes of cooking or after cooking if possible. It is recommended to avoid storing salt in hot and humid condition or near the cooking area (Tana and Raghuvanshi, 2013).

The study of heat effects by Bhatnagar *et al.* (1997) showed that only 18.5% of iodine was lost from iodized salt (potassium iodate used) in dry heating for $350^0$C by using iodometric titration methods (Bhatnagar *et al.*, 1997). However, Wang *et al.* (1999) stated that the iodine concentration loss was varied and dependant on the kind of food cooked where the retention of iodine ranged from 36.6% to 86.1% (Wang *et al.* 1999). Dasgupta *et al.* (2008) found that two samples out four have lost 10-20% of their iodine during exposure to 200˚C for 5 min. On the other hand, there were no noticeable losses for the others two samples (Dasgupta *et al.*, 2008).

## 4.4. Effect of light on iodized salt

For testing the effect of light on iodized salt package, which had 48.2 mg iodine/kg, two samples were taken; the first sample was kept in dark and another sample in light, in both glass and plastic container for 30 days. The iodine concentration was measured and calculated for each sample based on the results in (Table 15).Results indicate that the iodine concentration decreased to (21.9) mg/kg in dark in plastic and (22.4) mg/kg in glass, while it was decreased to (23.4) mg/kg in light in plastic and (24.1) mg/ kg in glass container.

The remaining of iodine in both salt samples showed that they were less than the Palestinian Technical Regulation recommended concentration. Comparing the percentage of loss iodine from salt in samples stored in glass container with that kept in plastic container are shown in Table 15.

Additionally, the questionnaire results showed that about 42.5% of consumers keep salt containers in the light, whereas, about 47.5% of them keep it in the dark.

**Table 15: Percentage loss of iodine level in salt, which was stored in dark and light**

| Type of container | % loss of iodine level | | | | |
|---|---|---|---|---|---|
| | Initial | In dark place | Net* loss in dark | In light place | Net loss in light |
| Glass container | 3.63 | 48.2 | 44.57 | 51.4 | 47.77 |
| Plastic container | | 49.7 | 46.07 | 52.7 | 49.07 |

* Net means the difference of % loss on dark or light and the % loss initial .

Liu's study (2007) showed that the iodine in the salt sample stored in ambient air and under light lost only slightly more iodine than the sample stored without light. Since iodine concentration decreased from 75 mg/kg to about 40 mg/kg after 42 days exposure to ambient air (Liu, 2007).Others studies showed that 11.94%-28.60% of iodine was lost upon exposure to sun light, and about 9.46%-21.40% of iodine was lost upon storage in cupboard for about 85 days.(Cynthia and Pelig-Ba, 2013)

In our study, aquestionnaire results showed that 32.3% of consumers stored salt in open containers, and 55.6% in closed ones. The rest (12.1%) did not care about keeping the containers closed or opened, so some times they kept it open, other times closed.

## 4.5. Analysis of the homogeneity of iodine in iodized salt

For each salt brand, the distribution of iodine in the three stages (top, middle, and bottom) was tested for homogeneity. Upon analysing iodine concentration in the different brands names, we found that most brands were almost homogenous brand. Table 16 shows the iodine concentration, average, standard deviation and the relative deviation for each salt brand.

**Table 16: The concentration of iodine level in different salt brand**

| Brand of salt name | Concentration of iodine (mg/g) | | | Mean | Standard deviation (SD) | Relative standard deviation (RSD) |
|---|---|---|---|---|---|---|
| | Top | middle | Bottom | | | |
| Saray salt | 0 | 0 | 0 | 0 | 0 | 0 |
| Al shilleh | 0.0212 | 0.0222 | 0.0219 | 0.0217 | 0.00054 | 2.39 |
| Salit | 0.0187 | 0.0187 | 0.0183 | 0.0185 | 0.0002 | 1.26 |
| Saleet | 0.0181 | 0.0183 | 0.0182 | 0.0182 | 0.00012 | 0.69 |
| Pure salt | 0.0184 | 0.0160 | 0.0093 | 0.0146 | 0.0047 | 32.28 |
| Al safy salt | 0.0290 | 0.0278 | 0.0229 | 0.0266 | 0.00321 | 12.08 |
| Sandy salt | 0.0196 | 0.0192 | 0.0165 | 0.0185 | 0.00168 | 9.12 |
| salt ملح | 0.0257 | 0.0268 | 0.0299 | 0.0275 | 0.00218 | 7.93 |
| salt | 0.2977 | 0.2996 | 0.2611 | 0.2861 | 0.02170 | 7.58 |

Results in Table 16 showed that “Salt” brand homogeneity is less than the others are, while saleet salt brand is the highest.

Dasgupta *et al*. (2008) studied the homogeneity in a single can of salt, in which since iodine was added to table salt as KI in dry form or spray form a solution. The researcher found that the iodine content varied in 5 samples taken from the same container of different depths (Dasgupta *et al*., 2008).

By studying the relationship between the date of production and the percentage loss, it was found that the iodine loss increased as a result of increasing shelf time increased for most salt brands. Out of the 12 brands collected, three were not iodized. While in the remaining nine, they were iodized (Figure 2), the percentage of iodine loss ranged from 38.1% to 100%

Limitations among the difficulties in this study were found, whereas some brands of salt have two concentrations of potassium iodate written on the bag.

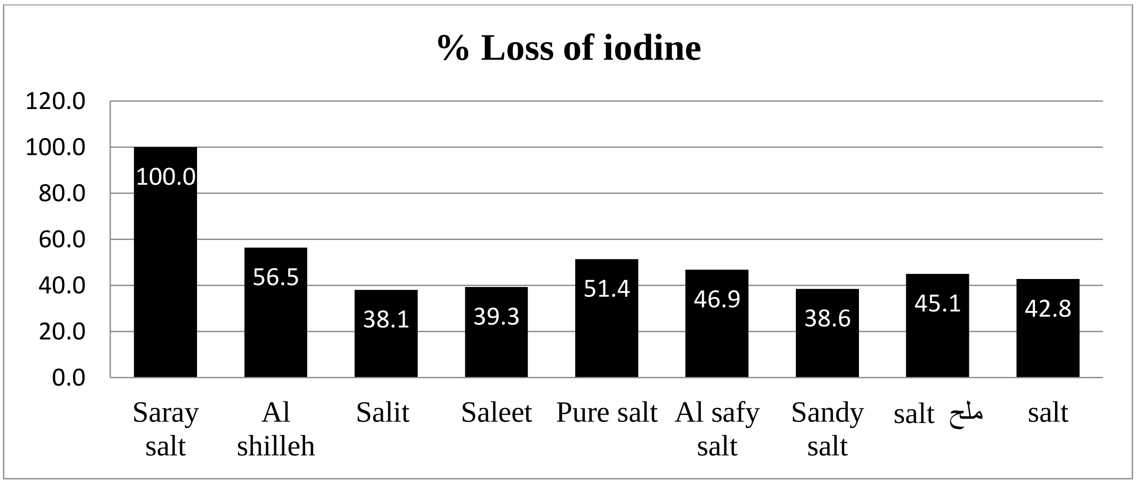


**Figure2:** The percentage of the concentration average lost for different brands

## 4.6. Development of the method of iodine analyses

When using the same amount of MB as the previous study of Narayana *el at*. (2007) the results showed that 0.5 ml of MB was not enough to react with the liberated iodine formed as a result of the reaction of iodate with iodide in acidic medium. So the liberated iodine selectively bleaches the blue color of methylene blue. The $R^2$ values for the unmodified and the calibration equation procedure was shown in the Table 17 for many sets of standards. The $R^2$ values range were from 0.52 to 0.70.

**Table 17: Calibration results of the unmodified procedure for a set of standards**

| Experiment # | Calibration equation | $R^2$ |
|---|---|---|
| 1 | y= 0.3116x + 0.1158 | 0.64 |
| 2 | y= 0.1452x + 0.1222 | 0.52 |
| 3 | y= 0.1757x + 0.1487 | 0.52 |
| 4 | y= 0.2669x + 0.087 | 0.70 |
| 5 | y= 0.1281x + 0.116 | 0.46 |
| 6 | y= 0.1276x + 0.095 | 0.56 |
| 7 | y= 0.1658x + 0.1284 | 0.54 |

The modification was done  by using 3 ml of MB instead of 0.5 ml. Using the modified method gave a better calibration results, $R^2$ values range  0.92 to 0.98. Thus, the modified procedure is adopted in performing our investigation .as shown in Table 18and Figure 3.

**Table 18: $R^2$ results and Calibration equations of modified procedure for set of standard**

| Experiment # | Calibration equation | $R^2$ |
|---|---|---|
| 1 | y = 0.9878x + 0.0728 | 0.98 |
| 2 | y = 1.1036x + 0.1748 | 0.93 |
| 3 | y = 1.0145x + 0.1146 | 0.95 |
| 4 | y = 1.1612x + 0.0592 | 0.98 |
| 5 | y = 1.0131x + 0.1276 | 0.95 |
| 6 | y = 0.8407x + 0.0833 | 0.97 |
| 7 | y = 0.9708x + 0.1159 | 0.96 |
| 8 | y = 0.8963x + 0.1339 | 0.94 |
| 9 | y = 1.0455x + 0.1472 | 0.94 |
| 10 | y = 1.0532x + 0.0968 | 0.98 |
| 11 | y = 1.049x + 0.1057 | 0.96 |
| 12 | y = 1.0862x + 0.0938 | 0.97 |
| 13 | y = 0.889x + 0.13 | 0.93 |
| 14 | y = 1.1433x + 0.1324 | 0.96 |
| 15 | y = 1.0774x + 0.1681 | 0.93 |
| 16 | y = 1.0282x + 0.1759 | 0.92 |
| 17 | y = 0.8328x + 0.0113 | 0.98 |
| 18 | y = 0.8329x + 0.0908 | 0.95 |

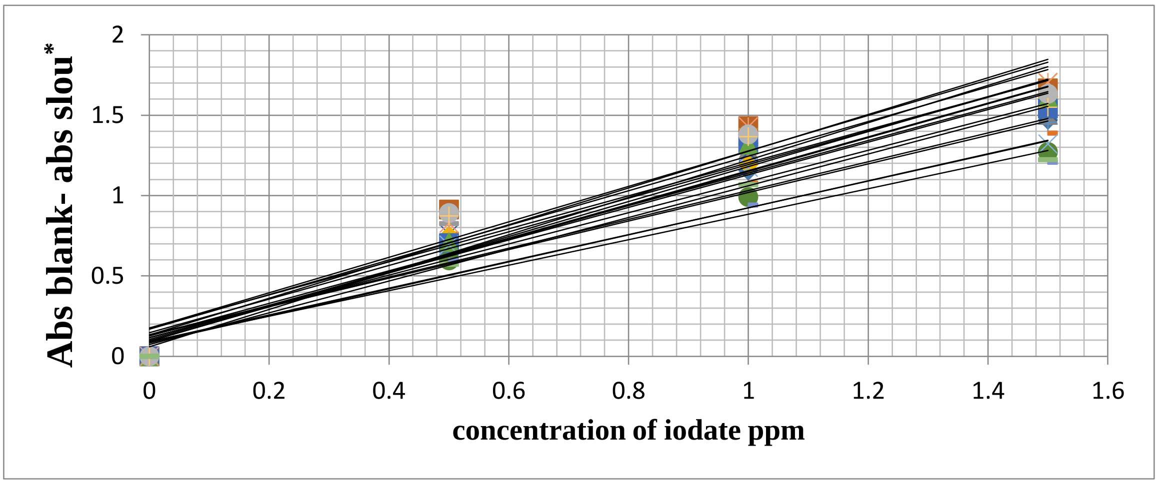


**Figure 3.** Calibration curve for the modified procedure for eighteen experiments

* The difference of the absorbance of blank solution and the absorbance of iodine solution.

# Chapter Five

# Conclusion and recommendation

## 5.1. Conclusion

According to the study results, the researcher sums up the following results:

1. The iodized salt in the market has different concentrations of potassium iodate according to salt source (50mg/100g, 50mg/1kg and 3mg/100g).
2. From the collected samples, about 9% of the samples have lost more than 80% of the iodine from iodized salt, while 23-28% of samples have lost 61-80% of iodine. On the other hand, not all remaining iodine where in compliance with the Palestinian Technical regulation. Thus, the result shows that only 43.5% of collected samples fit the Palestinian standards and 58 % fit the UNICEF ones.
3. The iodized salt was affected by light, which caused it to lose 47.8-49.1% of its iodine when exposed to light.
4. Heating salt via dry or wet methods caused a loss in its iodine content; dry heating could cause a loss of up to 26%, whereas wet heating caused a loss of 68%.
5. The concentration of iodine was homogeneous in most of salt brands; however, the concentration of iodine was zero in one salt brand.

## 5.2. Recommendations

According to the study results, several recommendations have been suggested:

1. Raising awareness about the importance of using iodized salt and iodine for human health by education and communication strategies. This should be done in order to improve knowledge about causes of IDD. Also increasing awareness about eating healthy, balanced and varied diet which probably provides enough iodine.
2. Notification for consumers about the proper salt storage conditions regarding light, temperature and heat.
3. Iodine content in salt packages have to be monitored in the Palestinian market.
4. Palestinian Ministry of Health has to monitor iodized salt and the potassium iodate concentration in it.
5. Further studies should be carried out in order to investigate factors affecting iodine concentration in salt.

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# Appendix A

- **The result of questionnaire paper**

| Paragraph of question | Number of people | % |
|---|---|---|
| Individuals use glass bottle | 41 | 41% |
| Individuals uses plastic bottle | 35 | 35.4% |
| Individuals use other (metal , same packet) | 23 | 23.2% |
| Israeli salt used | 49 | 49.5% |
| Palestinian salt used | 48 | 48.5% |
| Other resource of salt | 2 | 2% |
| Salt close to heat source | 25 | 25.3% |
| Salt far from heat | 74 | 74.8% |
| Salt put beside the light place | 42 | 42.4% |
| Salt put in dark | 47 | 47.5% |
| Salt bottle usually opened | 32 | 32.3% |
| Salt bottles usually closed | 55 | 55.6% |
| Salt bottle sometimes opened | 12 | 12.1% |
| Person who use more than 1 kg /month | 23 | 23.3% |
| Person who use less than 1kg /month | 76 | 76.8% |
| Person who had thyroid gland problems | 4 | 4% |

- **Table of different salt brand in Palestinian market**

| | Brand of salt | Name brand of salt | Conc. Of $KIO_3$ |
|---|---|---|---|
| 1 |  | SHILLEH TRADING Co.Ltd<br>Finest table salt<br>Nablus | 5mg/100g |
| 2 |  | Salit سليت<br>Finest table salt<br>Iodized<br>شركة الملح الإسرائيلية م.ض . عتليت | 3mg/100g |
| 3 |  | Salit<br>Table salt<br>Salt of the earth Ltd<br>عتليت | Not iodized salt |
| 4 |  | Saleet ساليت<br>Finest table salt Iodized<br>Market Place Co.Food substances Importation | 3mg/100g |
| 5 |  | Salt<br>Finest Table salt<br>Fine back Jeruslem | 50mg/100g |
| 6 |  | ملح الصافي<br>Dead sea Salt<br>مسودي للمواد الغذائية ولتجارة وتعبئة المواد الغذائية<br>Hebron ,Alshlalla St. | 50mg/kg |
| 7 |  | Saray Tuz salt<br>Iodized<br>Turkey<br>[http://www.saraytuz.com/](http://www.saraytuz.com/) | 20-40mg/kg |
| 8 |  | Sandy salt<br>Abu Zeineh Trading Co.<br>Hebron–Alsalam St.<br>انتاج شركة أملاح البحر الميت | 3mg/100g |

| | | | |
|---|---|---|---|
| **9** |  | **SALT ملح**<br>**Finest table salt**<br>**تعبئة وتوزيع مسودة الخليل**<br>**ملح طعام مدعم باليود** | **50mg/kg** |
| **10** |  | **ملح طعام نقي**<br>**Pure salt**<br>**Mawared National Co.**<br>**Hebron- Abu kteelh St.** | **3mg/100g** |
| **11** |  | **Shufersal** | ***** |
| **12** |  | **Lo salt**<br>**The orginal**<br>**From Scotland**<br>**Producted by Klinge Foods** | **** |

# Appendix B: Field Questionnaire

**هذه الاستبانة تهدف لدراسة "مستويات اليود في ملح الطعام المستخدم في المنازل "، وهي عبارة عن أسئلة تتناول كيفية استخدام والتصرف بالملح في البيوت.**

**علماً بان الاجابة عن الاسئلة بهدف البحث الاستفادة العلمية فقط. وان المعلومات هي سرية ولن يتم تناول اي ذكر أي اشخاص او اسماء.**

***A* الرجاء الاجابة عما يلي:**

**1. مكان السكن: المحافظة ................ المدينة .................. القرية................**

***B* يتم الإجابة عن الأسئلة من خلال وضع اشارة حول الاجابة المناسبة لوضعكم**

**2. الملح المستخدم في المنزل هو من إنتاج:**

**أ. اسرائيلي          ب. فلسطيني          ج. مستورد، حدد ............................**

**3. الكمية التي تشتريها هي:**

**أ. أقل من 1كغم     ب. 1كغم     ج. أكثر من 1 كغم الى 10كغم     د. أكثر من 10 كغم**

**4. يتم حفظ الملح لديكم في:**

**أ. عبوة بلاستيك          ب. عبوة زجاج          ج. عبوة معدن**

**د. بنفس الباكيت          هـ. أخرى، حدد............................**

**5. يتم حفظ الملح بالمطبخ:**

**أ. بالقرب من مصدر حرارة          ب. بعيد عن مصدر حرارة**

**6. يتم الاحتفاظ بالملح أيضاً:**

**أ. بالقرب من مصدر الضوء (الشمس) ب. بعيد عن مصدر الضوء**

**7. العبوة التي يحفظ بها الملح في أغلب الاوقات:**

**أ. تكون محكمة الأغلاق حتى الاستخدام ب. مفتوحة معظم الوقت ج. مفتوحة احياناً ومغلقة أحيانا أخرى**

**C* الرجاء الاجابة عن الاسئلة الآتية بلغة واضحة حسب الوضع لديكم:**

**8. كمية الملح المستهلكة شهرياً بالتقريب: ....................................**

**9. في حال كان الملح الذي يشترى أكثر من 1كغم، اين يتم حفظ الكمية المتبقية؟**

**.........................................................................**

**10. هل يعاني أحد أفراد الأسرة من مشاكل في الغدة الدرقية (الموجودة أمام الحنجرة)؟**

**.........................................................................**

**11. تاريخ انتاج الملح الذي اخذت منه العينات: ..........................**

**12. تاريخ انتهاء الملح الذي اخذت منه العينات: ..........................**

**13. ارجو كتابة أية بيانات إضافية كتبت على علبة/بكيت الملح الذي تم استخدمه لأخذ العينات .**

**جامعة النجاح الوطنية**

**كلية الدراسات العليا**

# تحديد مستويات اليود في ملح الطعام من الانتاج وحتى الاستهلاك في فلسطين

**إعداد**

**رندة طالب هاشم رجبي**

**إشراف**

**د. مآثر صوالحة**

**أ.د. أنسام صوالحة**

**قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس–في فلسطين**

**2016**

**تحديد مستويات اليود في ملح الطعام من الانتاج وحتى الاستهلاك في فلسطين**

**إعداد**

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**د. مآثر صوالحة**

**د. أنسام صوالحة**

## الملخص

يعد الملح المدعم باليود (ملح مؤيدن) من أفضل الطرق المستخدمة للوقاية من على مرض اضطرابات نقص اليود (IDD) وأمراض الغدة الدرقية. تتم اضافة اليود لملح الطعام اما على شكل الايودات أو الايوديد. لا توجد دراسات سابقة أجريت في فلسطين تقيس تراكيز اليود في ملح الطعام المستهلك لدى الأفراد او تراكيز اليود في عبوات ملح الطعام. يتأثر الملح المدعم باليود بالضوء والحرارة والزمن والعديد من المتغيرات. فقد تم جمع 99 عينة لهذه الدراسة من المحافظات الفلسطينية بشكل عشوائي وقد وجد أن 23–28 % من العينات خسرت ما نسبته 61 – 80 % من محتواها من اليود بينما وصلت نسبة الخسارة الى 100% في 9% من العينات. كما وبينت الدراسة أن حوالي 43.5 % من العينات كانت تتناسب مع توصية اللائحة الفنية الفلسطينية التي تنص على احتواء الملح على 35–55 ملغم لكل كغم، بينما 58% من العينات كانت تتناسب مع توصيات UNICEF (15ملغم/كغم). وقد لوحظ عدم وجود اختلاف في تراكيز اليود في العبوة خلال الاستهلاك. كما وبينت الدراسة تأثير الحرارة على تراكيز اليود في ملح الطعام حيث وصلت نسبة الخسارة في تراكيز اليود الى 67.5 % نتيجة الغليان، بينما وصلت الى حوالي 26 % بسبب التسخين الجاف. كما بينت الدراسة ان خسارة اليود في ملح الطعام المعرض الضوء أكبر في منه في العتمة. وقد وأظهرت الدراسة أيضا ان حوالي 9 من 12 من الانواع التجارية لملح الطعام في السوق الفلسطينية كانت من الأملاح المدعمة باليود. بينت كذلك ان اغلب العينات كانت متجانسة من حيت تراكيز اليود في داخل العبوة الواحدة، حيث تراوح مقدار الانحراف المعياري بين العينات

(أولها وسطها وأسفلها $1.25x10^{-4}$الى $2.17x10^{-2}$ ملغم/غم. حيث توصي الدراسة المستهلكين ومتخذي القرار والباحثين بالاهتمام أكثر بهذا الجانب.