Investigation of the Transformation and Effect of Preparation Method, Storage Conditions and Time on Iodine and Iron (II) Present in the Reed Salt

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Abstract: ***Background**: Salt, chemically known as sodium chlorideis the most commonly used food additive that improves the taste of foods and inhibits the growth of microorganisms. Despite the numerous diseases linked to the consumption of salt like hypertension, cardiovascular and kidney diseases its consumption has still continued to increase. Plant-derived foods including food additives like reed salt have the potential to provide human-essential minerals. Over one third of the world's population suffers from iron and iodine deficiency disorders withwomen and young children in the less developed countries being faced with moreserious health consequences.In Western Kenya, plant derived indigenous salts from Cyperus papyrus and Typha latifolia reeds have been usedfor cooking, medicinal purposes and a vast array of other uses despite lack of knowledge on its iodine and Fe^{2+} composition. **Objective**: The stability of iodine and iron (II)in the indigenous salts obtained from Cyperus papyrus and Typha latifolia reed plant species as affected by thepreparation methods, packaging materials storage conditions and time has been investigated. **Methods**: Iodometric titration and 1, 10-phenanthroline were used to determine the concentration of Iodine and Iron II in the salts respectively. **Results**: There was more loss of Iodine and iron (II)from the reed salts at elevated temperature and RH (82-100 % and 81-100 %) than at normal conditions of temperature and RH(72-100 % and 78-100 %), higher than the ICCIDD/WHO/UNICEF levels.Iodine was higher in Cyperus papyrus (7.2±0.423 - 34.5±2.327 mg/kg)than in Typha latifoliareed salt (2.3±0.214 - 4.4±0.212 mg/kg) when prepared using complete evaporation. Iron (II)reduced more with use of complete evaporation method than with evaporation crystallisation method. In respect to the packaging materials, the order of loss of iodine and iron II was LDPE<HDPE< banana leaves < open container and closed container for both methods. **Conclusion**: Iodine and iron (II)lossesdepend on plant species,method of preparation, packaging material and storage period and conditions. Cyperus papyrus salt prepared by complete evaporation method and packaged in aLDPE is recommendedwith a 3 months storageperiod.*

Keywords: *Typha latifolia*, *Cyperus papyrus,* indigenous reed salt, iodine and iron II

1. Introduction

Salt, chemically known as sodium chlorideis the most commonly used food additive that improves the taste of foods and inhibits the growth of microorganisms. Despite the numerous diseases linked to the consumption of salt like hypertension, cardiovascular and kidney diseases its consumption has still continued to increase (Shawel*et al.,* 2010). Many consumers and especially in Africa have had a preference of vegetable salt from ash obtained by burning plants parts which is potassium rich. In Central Africa, including Kenyan regions, vegetable salt is still traditionally produced and consumed either by alimentary habit or for therapeutic reasons; for atrophic gastritis, for icterus, to lower the blood pressure and as sedative against cough (Allaramadji, 2011).

In 2000, the World Health Report identified iodine, iron, vitamin A and zinc deficiencies as being among the world's most serious health risk factors. Iron deficiency anemia and iodine deficiency are two major nutrition-related disorders, affecting more than one third of the world's population (Shawel*et al.,* 2010). In the less developed countries, these deficiencies have serious health consequences, especially for women and young children. Iodine is an essential trace element that forms an integral part of the thyroid hormones which play an important role in controlling the rate of basic metabolism and in reproduction leading to iodine-deficiency disorders, which include anemia, goiter and a wide spectrum of mental and intellectual defects of varying degrees of severity, including cretinism, paralysis, and deaf-mutism (Fuge, 2007; Wisnu, 2008). On the other hand, low iron intake or poor absorption leads to anemia, resulting in a major reduction in work capacity and impaired immune response, which leads to a higher incidence of infection, increased risk of maternal and fetal morbidity, and a reduction in body growth. The combined impact of these deficiencies results in severe retardation of social and economic development of entire populations.

While plant-derived foods including plant-derived salts have the potential to serve as dietary sources of essential minerals for human use(Grusak, 2002), the traditional preparation of the vegetable salt is time consuming with low yielding quantity. The salt production method proceeds largely by lixiviation but depends on the region and the resulting brine is slowly dehydrated to obtain the dry salt (Gopalakrishnan, 2015). The plants used for preparation are mostly waste parts of cultivated plants like stems of maize, millet, sorghum and some widespread plants like *reed* species. The reports on chemical composition of vegetable salts are scarce. Zerries (1964) reported analyses of vegetable salts conducted by Martius in the Xingu river and by Sick in the British Guyana. Schmeda-Hirschmann (1994) analyzed four vegetable salts from the Papaguayan Chaco. Echeverri and Roman-Jitdutjaano (2011) have reported the chemical analyses of ash salts from 57 species used by the Witoto Indians of Amazon. Throughout the world more or less the same methodology has been practiced, but with little variations in the plants, tools and equipment utilized. Only little is known about ash salts from Africa and in this case western Kenya. This study considers vegetal salts due to their ease of preparation and wide spread utility. There is limited information on the chemical composition of the salt in regard to the availability and stability of iodine and iron in the reedsalts, the effects of storage conditions, packaging materials and the aging period as well as effect of storage conditions of temperature and humidity. Findings of this study have paid attention to the above gaps and its results are contained in this report.

2. Study Area

The study area comprised of two sites: Lugari and Busia regions of Western Kenya. Two sites were selected from each region, Matete and Lugari villages of Lugari division, in Kakamega region and Ululo and Bidimbidi villages in Matayos division of Busia region of Western Kenya (Figure 1). These regions were chosen based on the common method of processing of unrefined indigenous crystalline salts and the different reed species used in processing of the indigenous salts. Two types of papyrus reeds were used in this study; *Cyperus papyrus* reeds (Matayos) and *Typha latifolia* reeds (Lugari). Lugari district comprises of three administrative divisions Likuyani, Matete and Lugari, with a total area of 670.2 km^2 where Lugari and Matete divisions are selected in Lugari district for study. Lugari lies at an altitude of 1840 m above sea level records a mean annual rainfall range of 900-2200 mm falling within the upper midland (UM) and lower midland (LM) agro-ecological zone. Busia district in Western region covers an area of 1,261.3 km^2, 137 km^2 of which is part of Lake Victoria basin. Busia lies at 1220 m above sea level, has a rainfall range of 1300-1800 mm falling under lower midland agro-ecological zone (AEZ).The district is divided into six administrative divisions, namely Nambale, Butula, Funyula, Budalangi, Township and Matayos. Matayos division was randomly selected for this study in Busia district (Republic of Kenya, 2002-2008a). A Global Positioning System (GPS) was used to obtain the grid references of the location of the study sites.

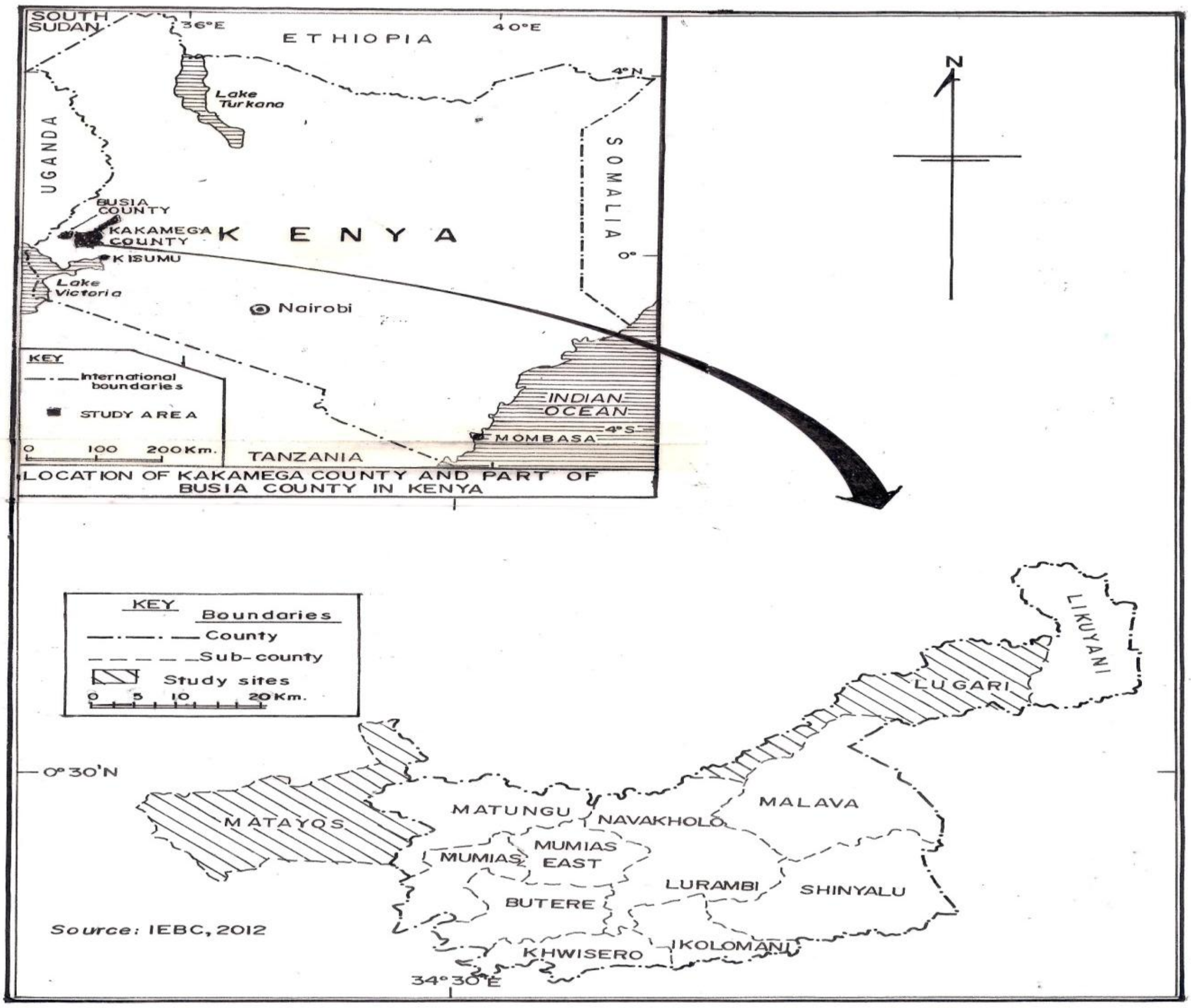

Figure 1: Map of Kenya Showing the sampling areas in Western Province

3. Materials and Methods

Chemicals, Reagents and Equipment

Double distilled water was used for dilution of reagents, preparation of both the samples and the working standards. All glassware and apparatus were cleaned by soaking in detergents for 24 hours and rinsed with double distilled water. Then they were soaked in 10 % HNO_3 for 24 h, rinsed with double distilled water and oven-dried at 110 °C. The standard solutions of the analytes used for calibration were produced by diluting the respective stock solutions of 1000 mg/L of the given element.

Sample collection and preparation

Samples of fresh reeds weighing 20 kg each were harvested in March, 2013 from each sampling sites; Ululo, Bidimbidi, Lugari and Matete; driedand burned to obtain ash. Filtration of the ash was done using a perforated plastic can. The filtrate was divided into two equal portions for further processing using the two methods of salt preparation. The first portion was boiled to complete evaporation in a steel open pan. Salt samples weighing 500 g each were collected and packed into polythene bags for further analysis. The second portion was exposed to evaporation crystallisation process to obtain a saturated solution. The saturated contents were packed into dried green banana leaves, tied well and placed into hot ash for drying to obtain the salt crystals. The salt samples were then unpacked and the granulated stones weighing 50 g each, were packed into five different polythene bags, for further analysis.

To test the effect of storage conditions, five packaging methods were used; woven high density polyethylene (HDPE) bags of 0.15 mm thickness; low-density polyethylene (LDPE) film bags of 0.07 mm thickness; open plastic; closed plastic containers and banana leaves packs. A set of storage conditions were selected as a representation of the extreme conditions applied for home storage, normal distribution and sale of salt in developing countries. To test the effect of aging, temperature and humudity on the concentration of Iodine and Iron, two sets of temperature and humidity were adopted: ambient temperature and humidity; (22 °C, 50 % RH) for analysis over 6 months and high temperature and humidity (40 °C, 100 % RH) for analysis of samples over a period of 18 days. For room temperature storage analysis; packages of the salts were sampled for analysis at the start of the experimental and after 1, 2, 3, and 6 months of storage. For accelerated temperature storage analysis; packages of the salts were sampled for analysis at the start of the experimental series (denoted as starting days in the results) and after 3, 6, 9, 12, 15 and 18 days. If the iodine content in the salt dropped below 20 ppm, no further analyses were performed on the sample. Below are results obtained with varying storage conditions, and time.

Analytical Methods

The pH values of the sampled salts were determined using a JENWAY pH meter model 3505 with a combined glass electrode (Ag/AgCl; PHE 1004), calibrated with pH 7 and pH 4.01 buffers, allowing 5 minutes for the reading to stabilize at a temperature of 20.8 °C. Determination of pH for each sample was done in triplicate.The moisture content was determined gravimetrically using an electronic analytical balance, Shimadzu type model AUY 120.

Iodine Analysis

Analysis of iodate content, IO_3^-, in the unknown indigenous plant salts was done using redox titration method (Iodometric method) (Abebe *et al.,*2012). This is based on the principle of reducing the iodate in the salt to free iodine, which can be titrated by sodium thiosulphate using starch as an indicator.

Iron II Determination

Phenanthroline method for total iron was used for determination of iron in the salt samples (Yadata, 2014). The ratio of ferrous to ferric iron was determined in the samples by titrating for ferrous iron with 1, 10-Phenanthroline(ACS grade), and determining the ferric iron by difference. The colour complex formed between the ferrous iron and 1, 10-phenanthroline ($C_{12}H_8N_2$) is an orange to red complex of ($C_{12}H_8N_2$) Fe according to equation 4.5. Each colour complex consists of three moles of 1, 10-phenanthroline and one mole of ferrous iron and it yields no colour with the ferric iron. Of the reagents used, standard iron Eisen standard was supplied by MERCK, Germany.

Determination of Cadmium, Chromium, Lead and Iron

To estimate the changes in bioavailability of iron, the amount of iron soluble in 1 M hydrochloric acid was determined using the wet digestion procedure (GüvenandAkinci , 2011; Charun and Farmer, 2006). The solutions were analyzed for iron directly by measuring their absorbance at 248.3 nm using a Thermo Jarell AshS11 Atomic Absorption Spectrophotometry (AAS) with hollow cathode lamps according to AOAC Official Method 3.6.1.2 (Okalebo*et al.*, 2002; Abebe *et al.,* 2012).

Statistical analysis

The data were based on three replicates and subjected to analysis of variance. Statistical analysis was done using GENSTAT Version 15.1. Means were calculated and compared using t-test , standard errors of each individual nutrient of the samples were computed, and variations among the species were evaluated by least significance difference (LSD) at 5 % level of probability ($p<0.05$). Stepwise regression was done for nutrients iodine, iron, magnesium, potassium and sodium to establish each independent variable's contribution to the change in iodine and iron contents of the salts.

4. Results and Discussion

Indigenous reed salts of *Typha latifolia* and *Cyperus papyrus* were analysed for pH and moisture content and for the presence of iodine, Fe^{2+} and Heavy metals Fe, Pb, Cr and Cd. The results are tabulated in the below.

pH and moisture content of salts

Both *T. latifolia* and *C. papyrus* salts were found to be alkaline with pH ranging between 9.6-10.1 which agrees with findings by Gopalakrishna (2015), who found the pH of traditional vegetable salts of Papua New Guinea to be in the range of 9.2-10.1 for seven types of salts.This observation clearly supports the presence of alkaline anions like hydroxide, carbonate among others. Complete evaporation method, retains more moisture than evaporation crystallisation method for both Busia and Lugari reed salts. However, the moisture content for Busia's *C. papyrus* salts was higher than Lugari's *T. latifolia*, with Bidimbidi having the highest (15.6 %), (**Error! Reference source not found.**). The presence of bicarbonate, carbonate, oxide and hydroxide ions (equations 8 to 10) makes the brine solution alkaline because solutions of alkali and alkaline earth chlorides and sulphates are expected to be neutral with a pH of 7. In fact, sulphates are also capable of decomposing during the ashing process Gopalakrishnan, (2015) as shown in equation 4. This also partly explains the deliquescent nature of some salts.

$$2\,HCO^{3-} \rightarrow CO_3^{2-} + H_2O + CO_2 1$$
$$CO_3^{2-} \rightarrow O^{2-} + CO_2 2$$
$$O^{2-} + H_2O \rightarrow 2\,OH^- 3$$
$$SO_4^{2-} \rightarrow O^{2-} + SO_3 4$$

Moisture isplays a key role in the stability of iodine, which then affects Fe^{2+}. The pH of the condensed moisture on the salt is influenced by the type and quantity of impurities present, and this may in turn affect the stability of the iodine compounds. Dampness causes moisture absorption of by the salt which slowly dissolves the iodine. To avoid this, a cool and well ventilated storage system is necessary to reduce the amount of iodine lost. This finding agrees with what Cynthia and Pelig-Ba (2013) observed where moisture affected the iodine content of Ghanaian salts.

Effect of method of preparation and type of reed species on iodine and iron stability in salt samples

Complete evaporation method retained more moisture (**Error! Reference source not found.**) and iodine (**Error! Reference source not found.** and **Error! Reference source not found.**) than using evaporation-crystallisation method. It is likely that some of the differences in nutrient composition are related to the differences in moisture content (Burlingame *et al.,* 2009). Ferrous compounds can be oxidized by iodate resulting in the loss of iodine, equation 5:

$$2\,I_5^+ + 10\,Fe^{2+} \rightarrow I_2 + 10\,Fe^{3+} 5$$

The elemental iodine forms by the reactions 6 and 7;

$$4Kl + 6O_2 \rightarrow 2\,I_2 + 2\,K_2O + 5O_2 \rightarrow 4\,KIO_3 6$$

and $2KIO_3 + 5H_2 \rightarrow I_2 + 5H_2O + K_2O \rightarrow I_2 + 4H_2O + 2\,KOH$ 7

Iodide oxidizes to form elemental iodine (5), and further under strongly oxidizing conditions to form iodate (equation 6). The iodate can be reduced to iodine (equation 7), and then, further reduced by a strong reducing agent to iodide (6). Typically both reactions stop at the formation of elemental iodine. Both reactions are favored by the presence of acidity, which shifts the equilibrium towards the production of free iodine. Moisture is required for these reactions to take place. The higher the moisture contents the more the reactivity.

The concentration of iodine in *Typha latifolia* salt ranged between 7.2±0.423 - 34.5±2.327 mg/kg and 2.3±0.214 - 4.4±0.212 mg/kg for salt samples prepared using complete evaporation and evaporation-crystallisation methods respectively. In Busia region the concentrations of iodine in *Cyperus papyrus* salt ranged from 291.9±2.540 - 309.6±5.180 mg/kg and 38.1±2.350 - 45.7±1.050 mg/kg for complete evaporation and evaporation-crystallisation methods are used respectively. It was observed that the concentration of iodine was higher in *Cyperus papyrus* reeds salt than in *Typha latifolia* reeds salt when complete evaporation method is applied.

Both reeds retain more iodine when complete evaporation method is used, there is a higher concentration of iodine in the end reed salt produced when *Cyperus papyrus* reeds are processed to give the salt at room temperature and relative humidity. There is more sublimation reaction with crystallisation process, hence more iodine loss.The iodine and Fe^{2+} are lower at higher % moisture content. Complete evaporation method gives salts with higher moisture content, and since moisture accelerates the decomposition of Fe^{2+} (equation 8) as well as the reaction of formation of iodine (equation 5), it is clear that the decrease of iodine and Fe^{2+} can be associated with the moisture levels. Iron is most bioavailable in the ferrous (Fe^{2+}) form. Through oxidation, ferrous iron is converted to the ferric form (Fe^{3+}):

$$Fe^{2+}\,(green) \rightarrow Fe^{3+}(orange/red) + e^- 8$$

This reaction is accelerated by alkaline conditions, oxidizing agents, including air, salt impurities, such as magnesium chloride and magnesium sulphate, and high humidity. *Typhalatifolia* and *Cyperus papyrus* salts were found to be alkaline (**Error! Reference source not found.**). This is expected to affect the decomposition of Fe^{2+} to Fe^{3+} (equation 8) in the two types of salts. Moisture naturally present in salt or abstracted from the air by hygroscopic impurities such as magnesium chloride acts as the reaction medium for the decomposition of added iodate.

Complete evaporation method showed more loss in Fe^{2+} than for evaporation crystallisation method just as observed in the case of iodine loss. However, there was more Fe in *Typha latifolia* reeds salt than in *Cyperus papyrus* reeds.

It was observed that *Typha latifolia* reeds salt had 3-16 % more Fe^{2+} in the salt samples obtained using evaporation-crystallisation method as compared to complete evaporation methodand at a pH 10. However, at the same pH 10, *Cyperus papyrus* reeds salt had 0-29 % more Fe^{2+} when complete evaporation method is used (Error! Reference source not found.). Fe^{2+} is therefore translocated more into *Typha latifolia* reeds salt than into *Cyperus papyrus* reed salt. In summary, there is more iodine and Fe^{2+} in salts prepared by complete evaporation than with evaporation-crystallisation method in all the samples for both *T. latifolia* and *C. papyrus* salts. *T. latifolia* salt has less iodine but more Fe^{2+} than *C. papyrus* salt in both the two methods used (Error! Reference source not found.).

Effect of packaging materials on iodine and iron (II) stability in Reed salt samples

Packaging materials used affected the levels of iodine and iron (II) in the salt samples when stored for over a period of six months. It was observed that by the third month of storage the loss in iodine was of the order LDPE<HDPE< banana leaves < open container and closed container. The LDPE retained upto 15 % of Iodine at three months of storage and 8 % at the end of the storage period (**Error!**

Reference source not found.). This agrees with Diosady and Alberti (1998) who observed that salts with stabilizers lost 33 % of their iodine on average after 12 months at 60 % relative humidity in LDPE while salts from Canada, India, Philippines and Senegal lost less than 15 % of the added iodine over the first six months even at 100 % relative humidity when stored in low density polyethylene bags (Abebe *et al*., 2012). It also agrees with Laar and Pelig-Ba (2013) who observed that the length and choice of storage greatly affects the iodine levels in salts for either iodated or non-iodated and found out that iodine losses over the period ranged from 10 % to 100 %. Since LDPE is very tight against water, but open for diffusion of gases e.g. oxygen and vapor, the absorption of these gases could have contributed to the reactions that led to reduced iodine and iron II concentrations inthe salt samples. The formation of the hypohalite ion (IO^-), equation 8, in neutral aqueous solutions of iodine is negligible. Iodine, I_2, reacts with water to produce hypoiodite, OI^-, equation 9. The position of the equilibrium depends very much upon the pH of the solution.

$$I_{2(l)} + H_2O_{(l)} \rightleftharpoons OI^-_{(aq)} + 2\,H^+_{(aq)} + I^-_{(aq)} \quad 8$$

and

$$I_{2(l)} + H_2O_{(l)} \rightarrow H^+_{(aq)} + I^-_{(aq)} + HIO_{(aq)} \quad 9$$

In basic solutions (such as aqueous sodium hydroxide), iodine converts in a two stage reaction to iodide and iodate, equations 10 and 11 respectively:

$$I_2 + 2\,OH^- \rightarrow I^- + IO^- + H_2O \quad 10$$

$$3\,IO^- \rightarrow 2\,I^- + IO_3^- \quad 11$$

The ionic reaction is particularly efficient in stretched LDPE, suggesting that the reaction cavities in the stretched polymer catalyze the formation of extended poly-iodide chain structures (Karlsen and Spanget-Larsen, 2009). HDPE had 4 % iodine while the rest had lost almost all at the end of the storage period. Though salts stored in HDPE containers behaved similarly to the open containers at 22 °C and 50% RH (**Error! Reference source not found.**) since the retained moisture initiated ionic reactions where iodine could have undergone a redox reaction, through oxidation of iodide to iodine leading to the slow loss of iodide content in the salt and which was more accelerated since the salt had no stabilizers. Equally, this phenomenon can be attributed to the solubility of the iodide in the moisture of the salt and its transportation to the surface by capillarity there by escaping by sublimation since it is volatile. Moisture plays a critical role in the stability of iodine (Laar and Pelig-Ba, 2013). Iodine formed can bind to many different substances, for example other halogens as well as react with the heavy metal impurities in the salt, for example reaction with lead ions in the salt, equation 12; **Error! Reference source not found.**

$$Pb^{2+} + 2I^- \rightarrow PbI_2 \quad 12$$

The percentage loss of iron II by different packaging materials was of the order: open container and closed container > HDPE > banana leaves > LDPE and 57 % of Fe^{2+} respectively at three months of storage and 1 % of Fe^{2+} (**Error! Reference source not found.**) at the end of the storage period. It can be observed that LDPE retained more Fe^{2+} than the HDPE containers and the rest of the packaging containers. HDPE bag is a better water proof, but permeable to oxygen and the migrated oxygen is responsible for the considerable loss of both iodine and Fe^{2+} during storage through oxidation-reduction reactions, being accelerated by catalysis of the storage container surfaces. The above findings however differs with the findings of Abebe *et al*., (2012) that showed, HDPE retaining more iodine from iodized salt better than LDPE. It is important to note that by the third month, the rate of loss of iodine from the salt samples was higher in HDPE container than in LDPE which could have been considered ideal during the first and second month (**Error! Reference source not found.**). This suggests that salts prepared from reeds should not exceed three months storage period since the salt will have no iodine. However, during storage there was a higher loss in the first month in both the two methods of preparation of the salts. These findings suggest that the LDPE is the most preferred method of storing indigenous reed salt which agrees with the findings of Diosady and Venkatesh (2015).

Error! Reference source not found. shows losses of Fe^{2+} from the reed salts. There was a more drastic loss with the open container compared to the rest. The trend of loss in Fe^{2+} was of the order open container > closed container > banana leaves >HDPE and LDPE. Salts in the open and closed containers, as well as banana leaves appeared to have absorbed more moisture and allowed in more air and contributed to the instability of iodine and Fe^{2+}. However, it was noted that by the end of the six months storage period, all the Fe^{2+} present at the beginning of the analysis had been lost by the salt samples in all the packages used. From these findings, LDPE can be considered a more superior packaging material for indigenous reed salts under normal conditions of temperature and relative humidity, for retaining Fe^{2+} and iodine (**Error! Reference source not found.** and **Error! Reference source not found.**). It provided a better moisture barrier hence lessening the effects of decomposition for both iodine and Fe^{2+}.

Effect of temperature and Relative humidity on the Stability of iodine and Fe^{2+} in Reed salt

In all cases, the samples stored under normal conditions of temperature and humidity lost iodine at a lower rate than those stored at elevated temperature and humidity (40 °C temperature and 100 % RH). After six months of storage at normal conditions of temperature and relative humidity, 25 °C and 50 % relative humidity, the loss ranged from 69 % to 99 %, which is higher than expected on basis of the ICCIDD/WHO/UNICEF tables (Diosady *et al.,* 1997). Under normal conditions of temperature and RH, *Typha latifolia* salt retained 18 % iodine when stored in LDPE as compared to the rest of the containers while *Cyperus papyrus* salt stored in LDPE material retained 13 % iodine retention as compared to the rest. At elevated temperature and humidity, almost all the salts lost upto 100 % of iodine with a retention order of: 11 % > 7 % > 5 % > 3% >0 % for LDPE > HDPE >Banana leaves >Closed container >open container. At elevated conditions of temperature and RH, storage of *Typha latifolia* salt in LDPE container retains 5 % more iodine than *Cyperus papyrus* salt. There was more drastic loss at normal conditions than at high temperature and humidity.

These results agree with Diosady and Venkatesh (2015) who also observed that salt samples stored at 40 °C and 100 % RH lost iodine at a higher rate than those stored at 60 % RH.

Diosady *et al.,* (1998) reported 30 % to 98 % loss of iodine from iodized salt of the original iodine when the salt was exposed to high humidity. Also, these findings are in agreement with the results of Abebe and Negussie (2012) who found high amount of iodine loss when iodized salt was stored at accelerated temperature (40 °C) and at high RH (70-100 %). The effect of temperature on the iodine levels suggest that heat affects the rate of iodine losses as rapid decreases were registered at higher temperatures. This result can be attributed to the exposed nature of the reaction vessel and the presence of heat at such high temperatures causing elemental iodine to readily sublime which is then lost to the atmosphere due to the high volatility of the element. The effect of temperature on the iodine levels suggest that heat affects the rate of iodine losses as rapid decreases were registered at higher temperatures and humidity. Equally, increase in humidity increases the amount of water absorbed by the salt as which apparently also increases with increased temperature. This in turn increases the redox reactions in the salt as well as decomposition reactions thereby losses in iodine content.

The rate of loss in Fe^{2+} at elevated conditions of temperature and humidity was of the order LDPE< HDPE < Banana leaves, Closed container and open container, with over 50 % loss. Of the packs, LDPE retained 45 % of Fe^{2+} in the salt at these conditions. Loss in Fe^{2+} was more pronounced with *Cyperus papyrus* salt than with *Typha latifolia* salt at 40 °C temperature and 100% RH (**Error! Reference source not found.**). There was upto 100 % (*Cyperus papyrus* salt) and 98 % (*Typha latifolia* salt) losses in Fe^{2+} by the 18th day of storage period at 40 °C temperature and 100 % RH (**Error! Reference source not found.**). By exposing the packaging containers to sunlight or in storage containers heated by the sun, high humidity would be retained once moisture is absorbed into the packaged contents, since the containers are permeable. The presence of moisture and permeability to oxygen by the containers provided conditions for oxidation of Fe^{2+} to Fe^{3+} hence the decrease in the concentration of Fe^{2+} Equation 13; i.e.

$$Fe^{2+}(green) \rightarrow Fe^{\,3+}\left(\frac{orange}{red}\right) + e^{-} 13$$

In the presence of increased moisture as a result of increased relative humidity, the effect of permeability is more felt, with more absorption of moisture by the salt. Fe^{2+} will undergo oxidation resulting in enhanced losses as increase in temperatures increases the rates of reactions. Polyethylene containers are known to be open for diffusion of gases e.g. oxygen and vapor, the absorption of these gases could have contributed to the reactions that led to reduced iodine and iron II concentrations in the salt samples. These ionic reactions are further catalyzed by reaction cavities (Karlsen and Spanget-Larsen, 2009) and at elevated temperature and humidity this reactions are more enhanced.

The losses of up to 100 % clearly indicate that effectively all of iodine and Fe^{2+} present in the salt had been lost within six months of storage at normal temperature and RH as well as within 18 days of storage at elevated conditions of normal temperature and RH (**Error! Reference source not found.** and **Error! Reference source not found.**). However, there was more loss of both iodine and Fe^{2+} at high temperature and relative humidity than at normal conditions. This demonstrates the large effect of high temperature and ambient humidity on the stability of iodine and Fe^{2+} and especially since the indigenous reed salt has no stabilizers, the loss was more drastic. Equally, LDPE was found to be more ideal for use as a storage material at elevated temperature and % RH, although from the findings it should be noted that storage should not exceed three days if elevated conditions are applied. The results of this study give an indication of the expected iodine and Fe^{2+} loss in the field. It is likely that the relative humidity and temperature may not necessarily remain at these extremes for six months.

Effect of storage time on the concentration of iodine and Fe^{2+} in the salt at 22 °C and 50 % RH

Both Iodine and Iron declined in concentration with time. It was observed that at the end of the six month period, most of these ions had been lost. It was noted that the type of reed plants had an effect on the pattern of ion loss in the salts.*Cyperus papyrus* salt lost more of the iodine present in the salt than *Typha latifolia* salt; its loss of iodine was more pronounced than for the later during the first two months of storage (**Error! Reference source not found.** and **Error! Reference source not found.**). However, after the two months *Typha latifolia* salt lost almost all iodine apart from two samples stored in HDPE (94 %) and LDPE (72 %) as compared to *Cyperus papyrus* that had between 85-98 % losses (**Error! Reference source not found.** and **Error! Reference source not found.**). It is thought that the absence of stabilizers coupled with the presence of moisture as well as high alkaline conditions contributed to the high losses in iodine. The loss of iron II is generally followed the same trend as Iodine from the 0th month to the 6th month with upto 100 % loss for all samples apart from the one stored in LDPE container. However, there was more loss in *Typha latifolia* salt between 0-3rd months (**Error! Reference source not found.**) as compared to *Cyperus papyrus* salt. While *Typha latifolia* salt lost between 68-100 % of Fe^{2+}, *Cyperus papyrus* salt lost all Fe^{2+} that was present in the initial salt sample. It is likely that the presence of moisture and impurities accelerated the loss of iodine from the salt.

Effect of Reed Plant Species

The effect of plant species on the retention of iodine and iron II in the indigenous reed salts of *Typha latifolia* and *Cyperus papyrus* salts was also investigated and findings reveal that *Cyperuspapyrus* salt proved to be more superior to *Typha latifolia*. It was observed that the concentration of iodine was higher in *Cyperus papyrus* reeds salt than in *Typha latifolia* reeds salt when prepared using the complete evaporation method (**Error! Reference source not found.** and **Error! Reference source not found.**). Also, it was observed that while using the same method of for the reed salt and at almost the same pH for the salt obtained, there was 44-88 % more loss of iodine in *Cyperus papyrus* reed salt as compared to 85-87 % iodine and in *Typha latifolia* reed salt samples. On the other hand, considering Fe^{2+} in the reed salt, there was no significant difference in method of preparing the salt since they displayed varying behavior the amount of Fe^{2+} loss. It was observed that with the *Typha latifolia* reeds, and at a pH of 10 for the salt obtained, there was 3-16 % more Fe^{2+} in the salt samples obtained using evaporation-crystallisation method as compared to complete evaporation method. However, with *Cyperus papyrus* reeds salt samples at pH 10, there was 0-29 % more Fe^{2+} when using complete evaporation method for preparation.

5. Summary, Conclusion and Recommendations

5.1 Summary, Conclusion

All the salts lost iodine and Fe^{2+} over the six months sampling period. The losses ranged from 78-100 % of the original Fe^{2+} and 72-100 % of the original iodine in the samples. However, at elevated conditions of temperature and RH; the losses were higher with 82-100 % and 81-100 % for iodine and Fe^{2+} respectively. The rate of loss of iodine and Fe^{2+} was influenced by the source of the salt, method of preparation of the salt, packaging material and storage conditions, temperature and relative humidity. It was also confirmed that the method of preparation of the reed salts has an effect on the salt's moisture, iodine and Fe^{2+} levels. *C. papyrus* salts had higher moisture content when prepared using complete evaporation method. The concentration of iodine was found to be higher in *Cyperus papyrus* reeds salt than in *Typha latifolia* reeds salt when prepared using the complete evaporation method. Both *T. latifolia* and *C. papyrus* salts were found to be both alkaline with a pH over 9.5. It was noted that evaporation crystallisation method contributed more to the loss of iodine from indigenous reed salt than the complete evaporation method. However, the concentration of Fe^{2+} on the other hand was found to reduce more with complete evaporation method than the salt prepared by the evaporation crystallisation method as observed for the case of iodine.

The type of packaging materials used affects the levels of iodine and iron (II) in the indigenous reed salt when stored over time. The loss in iodine and iron II was of the order LDPE<HDPE< banana leaves < open container and closed container for both complete evaporation and evaporation crystallisation methods. This was also observed at elevated conditions of temperature and RH, where iodine loss was of the order LDPE < HDPE < Banana leaves < Closed container < open container. The LDPE film provided a better moisture barrier retaining upto 8 % and 1 % of iodine and Fe^{2+} respectively at six months of storage. Effectively all iodine and Fe^{2+} present in the reed salts was lost within six months of storage at normal conditions of temperature and RH as well as within 18 days of storage at elevated conditions of temperature and RH. However, there were more losses at elevated temperature (40 ^{o}C) and relative humidity (100 %). After six months of storage at normal conditions of temperature and relative humidity, 25 ^{o}C and 50 % relative humidity, the loss was higher than the ICCIDD/WHO/UNICEF levels. The choice of the reed species is an important aspect to consider in obtaining an indigenous reed salt that would serve to provide adequate iodine and Fe^{2+}. *Typha latifolia* reed salt retains less iodine than *Cyperus papyrus* when the same conditions of packaging, temperature and relative humidity a well as method of preparation are applied. The level of heavy metals in both *Cyperus papyrus* and *Typha latifolia* was of the order Fe > Pb > Cd, while Chromium was not detected in all the samples. The concentrations of Fe, Pb and Cd in the salts exceeded the WHO/FAO permissible limits. *Typha latifolia* and *Cyperus papyrus* salts contain Fe Cd and Pb contaminants, with more Fe in Busia salts than in Lugari salts. The presence of more Fe impurity in *Cyperus papyrus* salts contributed upto 95 % loss in iodine as compared to 72 % loss in *Typha latifolia* salt for the LDPE packing material. The presence of these impurities contributed to the loss in iodine which eventually affected the amount of Fe^{2+}.

It therefore suggests that people in Western Kenya use indigenous reed salt that is highly contaminated with Fe, Pb and Cd while the Cr level is below the permissible limit. It was therefore established that the rate of loss of iodine and Fe^{2+} was influenced by the plant species, packaging material used for storage, method of preparation of the indigenous salt, storage period and conditions of temperature and relative humidity during storage. The results indicate that with careful control of these conditions, indigenous reed salt could be stabilized for a period not exceeding three-months from preparation. To maximize on iodine and Fe^{2+} availability in the indigenous reed salt, Cyperus papyrus salt from Busia prepared by complete evaporation method is recommended with LDPE storage for a period not exceeding 3 months.

5.2 Recommendations

Since the rate of iodine and Fe^{2+} losses was influenced by the plant species, packaging material used for storage, method of preparation of the indigenous salt, storage period and temperature and relative humidity during storage, it follows that with careful control of these conditions, indigenous reed salt could be stabilized for a three-month period for consumption. To maximize on iodine and Fe^{2+} availability in the indigenous reed salt, Cyperus papyrus salt prepared by complete evaporation method is recommended with LDPE storage for a period not exceeding 3 months.However, at elevated conditions of temperature and relative humidity banana leaves, closed plastic container or LDPE is best for storage for a period not exceeding 3 days.Storage of the salt in a dry place and a clean airtight plastic container while avoiding excess exposure to sunlight and heat could possibly reduce the level of post-production loss of iodine as well as Fe^{2+}. These findings call for the establishment of an effective monitoring and evaluation system of locally processed indigenous reed salts for iodine and Fe^{2+} levels to ensure consumption of standardized salt composition. Creation of awareness about proper storing and handling of indigenous reed salt may prevent possible loss of iodine through the production and storage processes. There is need for further studies to determine the actual concentration of iodine consumed at the household level to on guide whether there is need for fortifying the reed salt.

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