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Full Length Research

The stability of micronutrients in fortified food stuffs after processing and storage: Iodine in salt and iron in wheat flour

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Salt was fortified by potassium iodate in the level 66 mg/kg of salt with wet method of fortification. Then the iodized salt was stored using three packaging materials; low density polyethylene (LDPE), high density polyethylene (HDPE) and woven high density polyethylene (WHDPE) at two conditions: room temperature (20-25°C) and medium RH (50-60%) for about 6 months and accelerated temperature (40°C) and high Relative Humidity (RH) (70-100%) for about 18 days. Generally, the amount of iodine decreased with time ($p < 0.05$) but accelerated storage resulted in rapid loss of iodine. Among the three packaging materials used, HDPE retained iodine from iodized salt better than the other two packaging materials. In addition, a significant amount of iodine was lost from the iodized salt after processing the iodized salt at different temperatures. Wheat flour was fortified by ferrous sulfate in the level 40 mg/kg of wheat flour for room temperature (for 45 days) storage and 30 mg/kg of wheat flour for accelerated temperature (40°C) and high RH (70-100%) (for 4.5 days) storage. For both storage conditions iron was found to be stable ($p > 0.05$). When the amount of iron was evaluated after processing (baking bread), the result was found to be non-significantly different. In addition, the sensory quality scores of the 30 and 40 ppm iron fortified bread were above moderately liked scale and were not different from the bread made from the control.

Key words: Fortification, salt, wheat flour, micronutrients, iron, iodine, storage, relative humidity, temperature, packaging materials, processing, sensory evaluation.

INTRODUCTION

Sufficient micronutrients in the daily diet are one of the prerequisites for human health. Estimates suggest that some 815 million households worldwide suffer from micronutrient deficiency. To solve the problem of micronutrient deficiency, fortification of staple food has proven to be very efficient for certain micronutrients; the iodization of salt, fortification of wheat flour with iron, and fortification of vegetable oil with vitamin A (Poletti et al., 2004). Food fortification is the addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups (Clark, 1995). Relative to other modes of intervention, food fortification have been widely noted and a result it can be implemented and yield results within a short period. The focus of the international community has so

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far been on the three most prevalent deficiencies: vitamin A, iodine and iron (Johnson et al., 2004).

The iodine deficiency resulting from geologic rather than social and economic conditions cannot be eliminated by changing dietary habits or by eating specific kinds of foods but should be corrected by supplying iodine from external sources. It has, therefore, been a common practice to use common salt as a vehicle for iodine fortification for the past 75 years. Salt is consumed at approximately the same level throughout the year by the entire population of a region. Universal salt iodization is now a widely accepted strategy for combating Iodine Deficiency Disorder (IDD) (Bury et al., 1998). Salt iodization began in 1922 in Switzerland and has been implemented in many countries as the major mechanism for combating IDD.

A number of strategies are followed worldwide to combat Iron Deficiency Anemia (IDA) among vulnerable groups. Iron supplementation, dietary counseling, control of infectious and parasitic diseases and food fortification with iron are some of the strategies. In the past, main strategy was supplementation of pregnant women through hospitals. However, this strategy had very limited impact. When IDA is population wide and results from a combination of low iron intake and low bioavailability, fortification of wheat flour with iron offers a number of strategic advantages.

Therefore, among all the strategies used to deliver additional iron to humans, food fortification has the greatest potential to improve the iron status of the people (Lynch, 2005; Anjum et al., 2006).

Fortification of wheat flour has been practiced in the USA and in the European States for more than 50 years without any problems. The technology is also applicable in other countries where wheat is the main cereal staple. Rice, corn wheat and their derivatives, such as flours and meals, are preferred vehicles for fortification because most staple foods consumed in developing countries fall into this category. Iron can be added to flour to replace what is lost in milling or to reach a level higher than is found naturally in whole wheat. The criteria for selecting the form of iron to add, that is, the fortificant, include its bioavailability, effect on the quality of flour, effect on the color of flour and flour products, and cost (Nalubola and Nestel, 2000).

However, the success of a fortification program depends on stability of micronutrients and food to which it is added. Exposure of the fortificant to any of the physical and chemical factors including heat, moisture, air or light, and acid or alkaline environments during food processing, packaging, distribution, or storage affects its stability (Huma, 2004).

So far, a study on micronutrient stability (iodine in salt and iron in wheat flour) is not available in our country, Ethiopia. Therefore, this study could provide important information's about the stability of these micronutrients after processing and storage.

MATERIALS AND METHODS

The stability of the micronutrients, iodine and iron were assessed by applying Completely Randomized Design (CRD) using standard analytical techniques (Iodometric Titration and Atomic Absorption Spectrophotometry) respectively.

Study setting

The experiment was conducted in Food Science and Nutrition Research Laboratory of Addis Ababa University and in Ethiopian Health and Nutrition Research Institute (EHNRI).

Tools used for iodine analysis

Salt (fresh), potassium iodate (KIO_3) (food graded), packaging materials: LDPE (0.07 mm thickness); the water vapor transmission rate (WVTR) is 1.0-1.5 g/100 in^2/24 h, HDPE (0.15 mm thickness);WVTR is 0.3-0.5 g/100 in^2/24 h and WHDPE (0.15 mm thickness); WVTR is greater than 1.5 g/100 in^2/24 hr, Grinder (Mortar), Sprayer, Blender, Hygrometer, Drying Oven (Model: DHG 9055A), Hot plate (Wagtech RG13-4QD, UK), and Analytical Balance (LA 204, measure Tech) were utilized.

Tools used for iron analysis

Wheat, ferrous sulfate ($FeSO_4.7H_2O$) (food graded), Grinder, Mixer (ERWAKA-AR401), Analytical Balance (LA204, measure Tech), packaging material (WHDPE), Hygrometer, Drying Oven (Model: DHG 9055A), Muffle Furnace (Carbolite Aston Lane, Hope Shefield, S302RR, England), Atomic Absorption Spectrophotometer (Varian, Spectra-10/20, Australia), Baking Oven (Model name: Severin), and Hot plate (Wagtech RG13-4QD, UK) were utilized. All The chemicals used were brought from Sigma Aldrich Company.

Salt fortification by iodine

Salt was collected from Afdera (Afar). Afdera, is found in Afar regional state. It is located in the south east part of Ethiopia around 900 km away from Addis Ababa. Afdera is found 100 m below sea level and having temperature ranges between (45-52°C), this temperature is not the room temperature of Addis Ababa but for Afdera where the salt samples were collected. However, the room temperature of Addis Ababa where the research was conducted is 20-25°C. The salt sample was fortified by potassium iodate in the level 66 mg KIO_3/kg of salt using wet method of fortification (FMoH, 2010). The mixture was blended properly to ensure uniformity. Then the iodine content of the iodized salt samples was determined using the iodometric titration described by AOAC (1996) 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.

Sampling

1. For room temperature storage; Packages of iodized salt was sampled at the start of the experimental series (denoted as month 0) and after 1, 2, 3, and 6 months of storage.
2. For accelerated temperature storage; Packages of iodized salt was sampled at the start of the experimental series (denoted as day 0) and after 3, 6, 9, and 18 days of storage.

Calculation

$$\text{Mg/kg (ppm) iodine} = \frac{\text{Titration volume in ml} \times 21.15 \times \text{normality of } Na_2S_2O_3 \times 1000}{\text{Salt sample weight in g}}$$

Evaluating the stability of iodine after processing

In our country, Ethiopia "Watt" is prepared by adding the iodized salt with different ingredients and heated at different temperatures for different time intervals. In this study, the iodized salt was dissolved in water (10 g/50 ml water) and heated at different temperatures (40, 50, 60, 70, 80, 90 and 100°C) in a drying oven using covered Erlenmeyer flask for 1 ½ h (Chavasit et al., 2002; Winger et al., 2008). Then the iodine content was evaluated using iodometric titration described by AOAC (1996).

Wheat flour fortification by iron

Wheat was collected from five open markets of Addis Ababa. The wheat flour was fortified by ferrous sulfate in the level 40 mg $FeSO_4$/kg of wheat flour for room temperature storage and 30 mg $FeSO_4$/kg of wheat flour for accelerated temperature storage. These mixtures were blended properly to ensure uniformity using ERWAKA mixer. Then the iron content of the fortified wheat flour was determined using Atomic Absorption Spectrophotometer (AAS) described by AACC method 40-70 (1999).

Sampling

1. For room temperature storage: Packages of the fortified wheat flour were sampled at the start of the experimental series denoted as day 0 and after 15, 30, and 45 days of storage.
2. For accelerated temperature storage: Packages of the fortified wheat flour were sampled at the start of the experimental series denoted as day 0 and after 1.5, 3.0 and 4.5 days.

Calculation

$$\text{Mg/kg (ppm) iron} = \frac{(\mu g/ml - \text{conc. blank}) \times V\text{extract}}{\text{Sample weight (g)}}$$

Where; µg/ml is from the AAS reading.

The stability of iron following processing

Leavened bread using leavening agents (0.58 g yeast/100 g wheat flour) for about 4 h fermentation and unleavened bread (mixing wheat flour with water) were made at 250°C for 30 min by using baking oven from the fortified wheat flour (Olaoye et al., 2006), and then the iron content was determined using AAS described by AACC method 40-70 (1999).

Sensory evaluation

Appearance, aroma, taste, texture, scoop forming ability and over all acceptability of the three breads were evaluated with 10 sensory panelists comprising staff (EHNRI) and students from, Food Science and Nutrition program and Food Engineering Department of Addis Ababa University in standard sensory analysis booth at EHNRI following standard procedures (Eddy et al., 2007; Ouyoun et al., 2010). Panelists were trained in the use of sensory evaluation procedures and the meaning of the descriptive terms used according to (Eddy et al., 2007). Panelists were instructed to evaluate each sample in the following order: appearance, aroma, taste, texture, and over all acceptability. The three samples were presented turn by turn in identical containers, coded with three digit random numbers. A nine point hedonic scale with 1 representing the least score (dislike extremely) and 9 the highest score (like extremely) was used. Water was provided to rinse the mouth between evaluations and covered expectoration cups were also provided when panelists did not wish to swallow the samples (Olaoye et al., 2006).

Statistical analysis

Data obtained during stability studies to identify possible difference among the packaging materials, the storage time, the storage conditions and processing methods were analyzed with analysis of variance (ANOVA) and t-test. When P values ($P < 0.05$) were found significant, the means of each parameter were compared using the least significant differences (LSD) procedures of the SPSS, version 15.

RESULTS AND DISCUSSION

The stability of iodine at room temperature storage

The iodized salt was packed by the three packaging materials and stored at room temperature and medium RH up to 6 months. Tables 1 and 2 show the amount of iodine content in ppm.

According to Tables 1 and 2, there is a significance difference ($p < 0.05$) in the iodine content of the iodized salt due to the storage time, the packaging materials and their interaction. If the packaging materials have the capacity to absorb moisture, then the potassium iodate in the salt may be reduced to elemental iodine. Therefore, the elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion. From Table 2, we can also conclude that from the three packaging materials used HDPE retains iodine from iodized salt better than LDPE and WHDPE.

This result is in agreement with the result reported by (Diosady et al., 1998), different salt samples stored at 60 % RH and at room temperature. After six months of storage losses were ranged from 0 to 20%, and losses after 12 months averaged approximately 40%. Among the packaging materials used, WHDPE bags readily absorb moisture from the air and the iodized salt will thus release iodine as a vapor. WHDPE also allowed any condensed moisture to drip out of the bag in the form of a saturated salt solution containing iodate. Hence WHDPE lost more iodine within six months compared to HDPE and LDPE (Diosady et al., 1998).

Table 1. The effects of storage time on the stability of iodine (ppm) at room temperature (20-25°C) and medium RH (50-60%).

Storage time	Packaging materials		
	LDPE	HDPE	WHDPE
Starting month	39.87^e	39.87^e	39.87^e
At 1 month	38.81^d	39.66^d	37.33^d
At 2 months	36.91^c	39.30^c	35.85^c
At 3 months	35.85^b	38.92^b	35.435^b
At 6 months	30.88^a	33.84^a	30.35^a

$^{a-e}$ Any two means (n=2) in the same column not followed by the same latter are significantly different (p< 0.05).

Table 2. The effects of packaging materials on the stability of iodine (ppm) at room temperature (20-25°C) and medium RH (50-60%) storage.

Packaging materials	Storage time				
	Starting month	At one month	At two months	At three months	At six months
LDPE	39.87^a	38.81^b	36.91^b	38.85^b	30.88^b
HDPE	39.87^a	39.66^c	39.3^c	38.92^c	33.84^c
WHDPE	39.87^a	37.33^a	35.85^a	35.435^a	30.35^a

$^{a-c}$ Any two means (n=2) in the same column not followed by the same latter are significantly different (P < 0.05).

Table 3. The effects of storage time on the stability of iodine (ppm) at accelerated temperature (40°C) and high RH (70-100%).

Storage time	Packaging materials		
	LDPE	HDPE	WHDPE
Starting day (day 0)	39.13^e	39.13^e	39.13^e
At 3 days	38.92^d	39.13^d	38.59^d
At 6 days	37.01^c	37.54^c	36.48^c
At 9 days	36.17^b	37.01^b	35.32^b
At 18 days	32.25^a	34.89^a	31.73^a

$^{a-e}$ Any two means (n=2) in the same column not followed by the same latter are significantly different (p < 0.05).

The stability of iodine at accelerated temperature storage

The iodized salt was packed by the three packaging materials and stored at accelerated temperature and high RH up to 18 days. Tables 3 and 4 show the amount of iodine content in ppm.

According to Tables 3 and 4, there is a significance difference ($p < 0.05$) in the iodine content of the iodized salt due to the storage time, the packaging materials and their interaction. If the packaging materials absorb moisture from the air, then the potassium iodate in the salt may be reduced to elemental iodine. Therefore, the elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion.

The above result is in agreement with the study by (Diosady et al., 1998), one month exposure of seven samples of iodized salt to 40°C and 70-100% RH resulted in most of the samples in WHDPE bags losing more than 25% of the added iodine. Iodine losses over six months of storage in this study ranged up to 100%, indicating that within the 12-month trial period, essentially all of the iodine added to the sample disappeared from WHDPE bags, which are effectively permeable to the atmosphere and LDPE lost 15% of their iodine after 6 months. At high RH, the losses were much more significant. Seven of the samples lost half of their iodine content after only one month, and the average iodine retention decreased rapidly to 37, 10 and 1% after 3, 6, and 12 months, respectively. Another study by (Diosady and Albert, 1998) reported that, Canadian salt, which was of high purity, and contained very little moisture, or hygroscopic

Table 4. The effects of packaging materials on the stability of iodine (ppm) at accelerated temperature (40°C) and high RH (70-100%) storage.

Packaging materials	Storage time				
	Starting day (0 day)	At 3 days	At 6 days	At 9 days	At 18 days
LDPE	39.13^{a}	38.92^{b}	37.01^{b}	36.17^{b}	32.25^{b}
HDPE	39.13^{a}	39.13^{c}	37.54^{c}	37.01^{c}	34.89^{c}
WHDPE	39.13^{a}	38.59^{a}	36.48^{a}	35.32^{a}	31.73^{a}

$^{a-c}$ Any two means (n=2) in the same column not followed by the same latter are significantly different (p< 0.05).

Table 5. The mean iodine content of iodized salt after processing (boiling) for 1½ h at different temperatures.

Temperature (°C)	Mean iodine content after processing (ppm)
40	39.13^{g}
50	38.60^{f}
60	38.07^{e}
70	37.01^{d}
80	36.80^{c}
90	36.48^{b}
100	33.84^{a}

$^{a-g}$ Any two means (n=2) in the same column not followed by the same latter are significantly different (p < 0.05).

impurities were relatively stable. At low RH, the iodine losses were less than 10% during the first six month of storage period, and less than 25% after a year. With 100% RH the protection of the LDPE bag maintained iodine losses at less than 8%; while in the WHDPE, 63% of the iodine was lost.

The stability of iodine after processing

The iodized salt was dissolved in water (10 g /50 ml water) and boiled at different temperatures for 1 ½ h and the amount of iodine was evaluated. Table 5 shows the mean iodine content of the iodized salt after processing. The amount of iodine before processing was 39.13 ppm.

As it can be observed from Table 5, temperature was found to have a significant effect ($p < 0.05$) on the amount of iodine content in the iodized salt during processing. When the temperature increases, the amount of iodine lost from the iodized salt increases. That is, the temperature liberates elemental iodine from potassium iodate from the salt, when the temperature is high the rate of iodine libration is also high. Therefore, the elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion.

The results in Table 5 is in agreement with the study of (Diosady and Albert, 1998), where a sample of iodized salt was heated to 120°C, in a covered Petri dish lost up to 28.5% of added iodine after only 1½ h. A study by (Dasgupta et al., 2008) also reported that, boiling has an effect on the stability of iodine in iodized salt. When the boiling temperature increases the amount of iodine lost also increases. Another study by (Biber et al., 2002) also reported that much amount of iodine was lost during high temperature cooking.

The stability of iron at room temperature storage

The 40 ppm iron fortified wheat flour was packed by WHDPE and stored at room temperature (20-25°C) and medium RH (50-60%) for about 45 days. Table 6 shows the mean iron content of the 40 ppm iron fortified wheat flour within 45 days of storage.

As it can be observed from Table 6, storage time has no significant effect ($p > 0.05$) on the stability of iron in 40 ppm iron fortified wheat flour. But the addition of ferrous sulfate to wheat flour affected the iron content significantly. The amount of iron present in the control (unfortified wheat flour) was 44.12 ppm. When 40 ppm ferrous sulfate was added, the amount of iron increased to 55.51 ppm (99.5% pure $FeSO_4.7H_2O$ contains 20.33% Fe). Hence, the increase in iron content than the control was correlated with the addition of iron fortificant.

Retention of 97-100 % of iron fortified wheat flour during storage for 45 days has been observed by (Huma, 2004); where the total iron content, (75.08 ppm) at the starting day, decreased non-significantly to 74.12 ppm at the end of storage (45 days). (Rubin, 1975) cited in (Huma, 2004) also reported that, the change in the

Table 6. The effects of storage time on the total iron content (ppm) in 40 ppm iron fortified wheat flour at room temperature (20-25°C) and medium RH (50-60%).

Storage time	Mean iron content (ppm)
Starting day (day 0)	55.51[a]
Day 15	55.48[a]
Day 30	55.54[a]
Day 45	54.96[a]

[a-d] Any two means (n=2) in the same column not followed by the same latter are significantly different ($p > 0.05$).

Table 7. The effects of storage time on the total iron content (ppm) in 30 ppm iron fortified wheat flour at accelerated temperature (40°C) and high RH (70-100%).

Storage time	Mean iron content (ppm)
Starting day (day 0)	51.72[a]
Day 1.5	51.70[a]
Day 3	51.74[a]
Day 4.5	51.66[a]

[a-d] Any two means in the same column not followed by the same latter are significantly different ($p > 0.05$).

amount of iron in iron fortified wheat flour was non-significant during six months of storage.

The stability of iron at accelerated temperature storage

The 30 ppm iron fortified wheat flour was packed by WHDPE and stored at accelerated temperature (40°C) and high RH (70-100%) for about 4.5 days. Table 7 shows the mean iron content of the 30 ppm iron fortified wheat flour within 4.5 days of storage.

The analysis of Table 7 indicates that, storage time has non- significant effect ($p > 0.05$) on the stability of iron from 30 ppm iron fortified wheat flour. Storage of iron fortified wheat flour at accelerated temperature and at high RH do not affect the amount of iron content, rather causes oxidation of iron from (Fe^{2+}) to (Fe^{3+}) and reduces its bioavailability (Martinez-Navarrete et al., 2002; Huma, 2004). Sustain (2000) reported that, storage of iron fortified wheat flour at accelerated temperature and high RH do not affect the amount of iron content, rather affect the fat content.

The stability of iron following processing

Leavened bread (0.58 g yeast / 100 g wheat flour) for about 4 h fermentation and unleavened bread were made at 250°C for 30 min and the mean iron content was evaluated after baking. Table 8 shows the mean iron content of the leavened and unleavened bread.

As it can be seen in Table 8, baking was found to have a non-significant effect ($p > 0.05$) on the total iron content of the bread. According to the paired-samples t- test analysis, baking does not have a significant effect on the total iron content for both leavened and unleavened breads.

The non-significant change of iron during baking is believed to be due to the stability of iron during processing (baking). A study by (Huma, 2004) reported that, the amount of iron before baking (the flour) and after baking (the bread) changes non-significantly, rather the bioavailability increases. That is, the available iron in flour and in dough is approximately 80% of the total iron, which is approximately 20% lower than the available iron in the bread.

Sensory analysis of the bread

Appearance, aroma, taste, texture, scoop forming ability and over all acceptability of the three breads made from control, 30 ppm iron fortified and 40 ppm iron fortified wheat flour were analyzed using a nine point hedonic scale and the results given in Table 9 were obtained.

Conclusions

Potassium iodate from the iodized salt was easily reduced to elemental iodine, the elemental iodine readily sublimes and is then rapidly lost to the atmosphere through diffusion. Packaging materials, storage time, temperature and relative humidity play a critical role in the stability of iodine. When the storage time increases the amount of iodine lost also increases. The amount of iodine lost was high when the iodized salt was stored at accelerated temperature (40°C) and at high RH (70-100%). Among the three packaging materials used (LDPE, HDPE and WHDPE), HDPE retains iodine from iodized salt better than the others. Processing also affects the amount of iodine from the iodized salt. When the processing (boiling) temperature increases the amount of iodine lost also increases. Fortification of wheat flour with iron is a simple technology and can be applied in our country in order to combat Iron Deficiency Anemia (IDA). Different types of iron fortificants are used to fortify wheat flour in different countries, but in this study ferrous sulfate ($Fe_2SO_4.7H_2O$) were used. And it was found stable during the stability study. Overall, the attempt of producing iron fortified wheat flour was successful; the bread produced from 30 and 40 ppm iron wheat flour was found to be not significantly different from the bread made from the control and had very good (like moderately) sensory quality.

Table 8. The effect of processing on the total iron content (ppm) in bread made from iron fortified wheat flour.

Types of bread	Mean iron content before processing (ppm)	Mean iron content after processing (ppm)
Leavened bread	55.51^a	55.53^a
Unleavened bread	55.51^a	55.48^a

[a-b] Any two means in the same rows not followed by the same latter are significantly different ($p > 0.05$).

Table 9. The effects of iron fortification on sensory quality of breads.

Iron proportion in breads	Sensory qualities					
	Appearance	Aroma	Taste	Texture	Scoop forming ability	Over all acceptability
Control (unfortified)	7.7^a	7.2^a	7.1^a	7.4^a	8.0^a	7.5^a
30 ppm iron fortified	7.9^a	7.7^a	7.5^a	7.8^a	7.9^a	7.6^a
40 ppm iron fortified	7.9^a	8.1^a	7.3^a	7.1^a	7.8^a	7.6^a

[a-c] Any two means (n=10) in the same column not followed by the same latter are significantly different ($P > 0.05$).

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