

Fortification and Stability of Iodine in Bread to Mitigate Iodine Deficiency Disorder

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Abstract. The core objective of this study was to prepare iodized bread by using iodized salt and potassium iodide (KI) to fulfill Recommended Daily Intake (RDI) of iodine. Triplicate level of each fortificant was added in separate treatments in different concentrations with an aim that 2 slices of bread provide RDI of iodine. The prepared samples were analyzed for stability of iodine by spectrophotometric method and for sensory attributes by panel of judges. Results showed good retention of iodine in bread with 15-20% loss of iodine in final product after processing. A slight level of potassium was increased in treatments in which KI was used while other minerals profile was not affected by fortificants and showed no significant behaviour after examining the results statistically.

Keywords: fortification, stability iodized bread, iodine deficiency

Introduction

A balanced diet is indispensable for optimal functioning, metabolism, growth and development of human body. Carbohydrate, protein, lipid, vitamin and inorganic micronutrients are prerequisite of balanced diet. Trace elements play vital role in human body as being structural component of hormones, vitamins and cofactors of enzymes (Freeland-Graves *et al.*, 2014). Even the least deficiency of various vitamins and minerals can disturb the functioning of immune system and can cause serious diseases (Akhter *et al.*, 2004). Minerals are classified as macro and micro minerals. Micro minerals also known as trace elements are inorganic constituents present in all tissues and fluids of human body. Minerals however do not produce the energy but are significant for many physiochemical processes required to carry out normal life processes (Soetan *et al.*, 2010).

Iodine, trace mineral element, present in minute fraction in human body is radiologically indispensable element. In human body, thyroid gland is the main organ in which iodine is present and it has an effectual role in the production of thyroid hormones. These hormones highly influence the functioning of nerve tissues and muscles, circulatory system, intellectual physical development and heat energy regulation of body (Zimmermann, 2008). It impedes abnormal growth of bacteria in

stomach and can transmute allergic proteins entering in body to non-allergic by coating them. It also deactivates most of chemical toxins, all biological poisons and spread of cancer cells to secure body from dreadful diseases and infections (Freeland-Graves *et al.*, 2014).

Iodine is a fundamental micronutrient needed at all stages of human life but the pregnancy, fetal life and early childhood are the most important stages of requirement (Zimmermann, 2008). World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF) and International Council for Control of Iodine Deficiency Disorder (ICCIDD) recommended that preschool children should take 90 µg of iodine per day, for school children this value is 120 µg per day, 150 µg per day for 12 years and above while pregnant and lactating women should take 250 µg of iodine per day (Longvah *et al.*, 2013). Pregnant women have more need of iodine because of the increased synthesis of thyroid hormone, mandatory for the normal and healthy development of fetal brain and neurological network (Elahi *et al.*, 2009).

Deficiency of iodine is the public health problem all over the globe. Amongst the ten greatest challenges to global welfare, universal salt iodization is third one suggested by the participants of Copenhagen Consensus (Horton *et al.*, 2008). More than 1.88 billion people in

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the world have insufficient iodine level including 241 million children. People are prone to several types of risks due to iodine deficiency like neonatal hypothyroidism, endemic goiter, pregnancy loss, cretinism, infant mortality, growth retardation and intellectual impairment (Pearce *et al.*, 2013; Andersson *et al.*, 2012). In Pakistan, 70% people are afflicted with iodine deficiency disorder (IDD). The appalling instance of iodine deficiency in pregnant females is main reason that one third of infants in Pakistan have low birth weight (Zimmermann, 2011). During early infancy and fetal life, most drastic effect of iodine scantiness occur on the brain (IM, 2002). Hypothyroid people are less efficient and consequently, economy can be handicapped by reducing work output (Jooste and Zimmermann, 2008).

Different strategies are available and applicable to control and prevent micronutrient deficiencies including iodine deficiency disorders. Three of them are dietary diversification, supplementation and fortification for both targeted and untargeted population (Popovici *et al.*, 2006). Food fortification means the addition of mandatory micronutrient in processed and treated foods. Food fortification is a worthwhile method reinforces the approaches used to diminish and restrain the problem of micronutrient malnutrition (WHO and FAO, 2006). Fortification of iodine is widespread to control and prevent the adverse effects of less iodine intake (Rasmussen *et al.*, 2014). Sodium and potassium iodides and iodates are recommended additives for fortification of iodine in food. Level for their addition to salt may be equivalent to 25-65 mg iodine per kg salt (Thomson, 2009). Iodized salt also has been proven an excellent and effective mean for iodine fortification in many industrialized countries (De Benoist *et al.*, 2004). Iodized salt is used in breads, biscuits and breakfast cereals due to higher consumption of bakery products (Thomson, 2009).

Bread is an important source having vital diet components that may include iodized salt. Fortified bread not only provides nutritional minerals to our cells, but it has potential to dissolve, sanitize and clean toxic wastes from our body system. Salt is one of the few perfect fortificants for micronutrient fortification because it is among those foods that are globally consumed on regular bases at a fairly constant rate by almost all sections of a population regardless of their economic and social status (Zimmermann and Boelaert, 2015). Fortification of bread with iodine to combat iodine

deficiency disorders and to determine processing effect on iodine stability were main objectives in this research.

Materials and Methods

Procurement of raw material. Wheat variety i.e. "Faisalabad 2008" for bread making was selected and procured from Ayub Agricultural Research Institute, Faisalabad. Chemicals and reagents were procured from Sigma Aldrich.

Chemical evaluation of wheat flour. Wheat flour was evaluated for chemical composition according to AACC (2000) i.e., moisture content with method No. 44-15A, crude protein with method No. 46-10, crude fat with method No. 30-10, crude fibre with method No. 32-10 and ash content with method No. 08-01. For moisture content 3-5 g sample was taken in China dish, put in hot air oven at 100 ± 5 °C for overnight and moisture content was measured by calculating the weight loss. Wheat flour sample about 1-3 g digested with concentrated H_2SO_4 till light green colour and distilled after diluting with distilled water about 250 mL total volume. After distillation, the resultant solution of ammonium borate was titrated with 0.1N H_2SO_4 . This method gives the estimation of total nitrogen which was converted to protein by multiplying with 5.7 factor.

For fat determination, 10-15 g sample was wrapped in filter paper and washed with petroleum ether in Soxhlet apparatus. At the end, fat free sample was placed in hot air oven at 100 ± 5 °C for overnight and fat content was measured by calculating the weight loss. Fat and moisture free sample (1-3 g) was digested with 1.25% solution of H_2SO_4 and after washing with 1.25% solution of NaOH. The residues obtained after washing and filtration were charred and placed in muffle furnace at 550 °C for 5-6 h. The fibre was calculated by measuring the difference before and after placing in muffle furnace. For ash determination, 1-2 g sample was charred on direct flame and placed in muffle furnace at 550 °C for 5-6 h. The remaining residues were measured as total ash content of flour.

Minerals like iron (Fe), copper (Cu), potassium (K), zinc (Zn) and manganese (Mn) in flour was analyzed using Atomic Absorption Spectrophotometer (Varian AA240) as described in AOAC (2006). Sample (1-3 g) was digested in a mixture of HNO_3 : $HClO_4$ with 7:3 over hot plate. After digestion, sample was diluted with distilled water to make total volume 250 mL. Afterwards, sample were run on atomic absorption spectrophotometer.

Product development. For bread, a control and test runs with iodized salt and potassium iodide (source of iodine) were manufactured in the normal way in baking hall of National Institute of Food Science and Technology. Triplicate samples were prepared with each of the treatment as mentioned in Table 1. The breads were prepared according to the AACC (2000) straight dough method No 10-10B. The ingredients were mixed for 5-10 min in a Hobart A-200 mixer to form dough, afterwards the dough was molded, panned into 100 g pans, and proofed for 45 min at 95 °F (35 °C) and 85% R.H in proofer. The dough was baked at 220 °C for 22-25 min and sliced after cooling. The bread slices were packed properly in polythene bags stored under ambient conditions and were analyzed afterwards.

Analysis of product. Chemical composition of bread. Moisture, crude protein and crude fat content of bread were analyzed by AACC (2000) methods as mentioned above. Minerals like Fe, Cu and Zn of bread were analyzed using Atomic Absorption Spectrophotometer (Varian AA240) as described in AOAC (2006).

Iodine content. All bread samples were analyzed for iodine content in triplicate by a spectrophotometric method according to Moxon and Dixon (1980). Bread samples (1g) of each were taken in porcelain crucible, 2 mL of 1M potassium hydroxide solution and 1mL of 10% of zinc sulphate solution was added. Mixture was driven, completely on hot plate and the crucible was placed in muffle furnace at 450 °C for 1.5 h. The residue was dampened with zinc sulphate solution then again placed in furnace under same conditions. Subsequently, the cooled ash was transferred to centrifuge tube with 50 mLs of deionized (DI) water. The samples were then mixed with 0.4mL of 0.006M potassium thiocyanate solution and 1.6 mL of 0.2M ammonium iron (III) sulphate. At exactly 90 second intervals, 1mL of 0.3M sodium nitrite solution was added. The absorbance of

mixture was measured at 450nm after 20 min incubation. Iodine content was calculated using KI as a standard.

Sensory evaluation. The bread was prepared as above and subjected for sensory evaluation for appearance, colour, texture, flavor and taste by hedonic score system as described by Lawless and Heymann (2010). For sensory evaluation of bread, 20 panelists were selected covering faculties from teaching, research and extension wings. Panelists were of 24-40 years age with sound health and good sensory perceptions. The evaluation was carried out in a well-ventilated, odourless, and quiet environment.

Statistical analysis. The data obtained for each parameter was subjected to statistical analysis to determine the level of significance among the treatments and further values were finalized by applying means separation (Tukey-HSD) using SPSS (Statistical Package for the Social Sciences, version 10.0.1, 1999) according to the method described by Montgomery (2008).

Results and Discussion

Chemical composition of wheat flour. Chemical composition of wheat flour is shown in Table 2 and the proximate analysis of wheat flour shows average results. Moisture content of wheat flour was 9.73%, protein was 10.54% and fat content of flour was 1.37%. Fibre and ash contents were 2.33% and 1.67%, respectively. Flour sample contain Fe, Zn, Mn and Cu as 1.27mg/100g, 0.82mg/100g, 0.80mg/100g and 0.24mg/100g, respectively. The finding of the current study is congruent to the finding of Ahmad (2017); Pasha *et al.* (2009); and Zahoor (2003) who reported that values of moisture, protein, fat, fibre and ash were in the range of 8.92 to 11.68 %, 10 to 13.4 %, 1.09 to 2.52 % and 2.20 to 2.77 %, respectively in some Pakistani wheat varieties. Similar results regarding Fe, Zn, Mn and Cu have been obtained from the study of Khan *et al.* (2005) who reported that wheat flour contains 4.40 mg/100 g, 3.40 mg/100 g, 3.53 mg/100 g and 0.63 mg/100 g of the above mentioned minerals, respectively.

Chemical composition of bread. Bread proximate showed non-significant results revealing no effect of any treatment on the moisture, protein or fat content of bread. Baking always involves the loss of water from the 'raw' to the baked product therefore bread contains lower moisture content as compared to water added during processing (Cauvain and Young, 2000). Chemical composition of bread is presented in Table 3. In bread

Table 1. Treatment plan for iodine fortification in bread

Treatment	Iodized salt & potassium iodide (KI)/100g
T ₀	Control
T ₁	1g iodized salt
T ₂	2g iodized salt
T ₃	3g iodized salt
T ₄	0.546mg (546µg) KI
T ₅	0.818mg (818µg) KI
T ₆	1.090mg (1090µg) KI

treatments, moisture content shows slight decreasing trend from T₀-T₂ then increases in T₃ and T₄ again decreases slightly in T₅ and T₆. It may be due to minor deviations in processing conditions. Protein content in bread decreased than protein content of flour and shows decreasing trend in treatments. Malomo *et al.* (2011) supported the results of this research, used wheat flour of 15.47% protein content and used it for bread production. After production of bread they found decreased content of protein (11.96%) while fat increased in bread than wheat flour and shows results similar to Malomo *et al.* (2011). He used wheat flour of 2.60% fat content for bread production and after production of bread found increased trend of fat (4.29%). Best treatment T₅ contain moisture 30.31%, protein 8.72% and fat 1.88%.

Mineral analysis and their statistical behaviour showed that all of them were non-significantly affected by iodized salt and KI treatments used in bread except K content, which shows a significant result. Iron and manganese content in bread was less than previous findings of some scientists as Tuncel *et al.* (2014) observed 2.44mg/100g iron content in pan bread prepared with flour of wheat grain. Ragaee *et al.* (2006) examined 44 mg/100g of iron content in wheat flour. This was due to the reason of using wheat flour for bread making that was already low in iron and

manganese content. Zinc content assessed in bread was close to the previous researches as Tuncel *et al.* (2014) investigated 0.95mg/100g zinc content in bread prepared with wheat flour. Mean values of Cu in all treatments were very near about each other with highest content of 0.23 mg/100g and lowest of 0.22 mg/100g found randomly in different treatments showed that there was no significant effect of any treatment on content of Cu in bread. Level of K slightly raised in last 3 treatments in which KI was used as fortificant but that increase in level was only of some micrograms not exceeding its recommended intake level.

Iodine content. Iodine stability is the main issue now a days that is contradicting the struggles of iodine fortification, therefore iodine stability test was done that showed only 15-20% loss of iodine and actually depicts a high stability in terms of its need to overcome its deficiency consequences. Loss of iodine level in different treatments of bread is depicted in Table 4. Three levels of iodized salt were used in bread to prepare iodine fortified bread but iodized salt through bread supply only a little portion of required iodine because the addition of salt in bread is limited for best yeast activity and acceptable sensory characteristics. Maximum of 3% iodized salt used in bread (T₃) and after loss of iodine, two slices of that bread gave 37µg of iodine. KI in this sense gave the best result and is much effective

Table 2. Chemical composition of wheat flour

Treatments	Moisture	Protein	Fat	Fibre	Ash	Fe Zn Mn Cu K				
						(mg/100g)				
R ₁	9.9	10.59	1.33	2.33	1.68	1.272	0.824	0.792	0.234	96.2
R ₂	10.1	10.72	1.41	2.38	1.64	1.319	0.859	0.817	0.245	92.2
R ₃	9.2	10.33	1.38	2.29	1.7	1.221	0.776	0.779	0.224	88.4
Mean value	9.73± 0.472	10.54± 0.199	1.37± 0.404	2.33± 0.045	1.67± 0.031	1.271± 0.049	0.82 ± 0.042	0.796± 0.019	0.234± 0.011	92.267± 3.9

Table 3. Chemical composition of bread

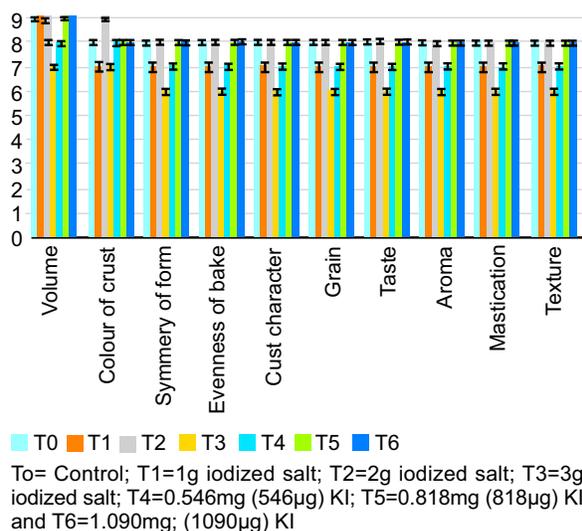
Treatments	Moisture	Protein	Fat	Iron	Zinc	Manganese	Copper	Potassium
T ₀	30.48 ± 0.93	8.71 ± 0.09	1.87 ± 0.09	1.26 ± 0.01	0.80 ± 0.01	0.78 ± 0.01	0.23 ± 0.01	89.27b ± 0.85
T ₁	30.14 ± 0.96	8.71 ± 0.07	1.91 ± 0.07	1.25 ± 0.01	0.81 ± 0.02	0.76 ± 0.01	0.22 ± 0.01	89.47b ± 0.97
T ₂	29.33 ± 0.99	8.72 ± 0.11	1.87 ± 0.08	1.26 ± 0.02	0.79 ± 0.01	0.78 ± 0.01	0.23 ± 0.02	90.3ab ± 0.91
T ₃	29.84 ± 0.99	8.69 ± 0.09	1.87 ± 0.11	1.26 ± 0.01	0.81 ± 0.02	0.76 ± 0.01	0.22 ± 0.01	91.3ab ± 0.82
T ₄	30.93 ± 0.67	8.69 ± 0.08	1.9 ± 0.08	1.25 ± 0.02	0.79 ± 0.02	0.76 ± 0.01	0.23 ± 0.01	91.3ab ± 0.83
T ₅	30.31 ± 1.11	8.72 ± 0.07	1.88 ± 0.11	1.25 ± 0.01	0.80 ± 0.01	0.77 ± 0.02	0.23 ± 0.02	92.3a ± 0.81
T ₆	29.43 ± 0.61	8.68 ± 0.09	1.89 ± 0.09	1.26 ± 0.01	0.79 ± 0.01	0.76 ± 0.01	0.22 ± 0.01	92.47a ± 0.71

Table 4. Loss of iodine level in bread

Treat-ment	Iodine	Fortificant added before processing per 100g	Iodine in bread after processing ($\mu\text{g}/100\text{g}$)
T ₀	1.99g \pm 0.01	–	–
T ₁	30.99f \pm 0.21	1g iodized salt (40 μg I)	30.99
T ₂	63.12e \pm 0.02	2g iodized salt (80 μg I)	63.13
T ₃	91.02d \pm 0.01	3g iodized salt (120 μg I)	91.02
T ₄	319.20c \pm 0.15	546 μg KI (417 μg I)	319.20
T ₅	479.87b \pm 0.15	818 μg KI (626 μg I)	479.87
T ₆	651.13a \pm 0.15	1090 μg KI (833 μg I)	651.13

to prepare iodized bread as T₅ bread treatment in which 0.818mg/100g KI was used, supplied 172 μg of iodine/ 2 slices.

Sensory analysis. In sensory evaluation of iodine fortified bread treatments volume was not altered significantly in all treatments as shown in Fig. 1. In T₂ and T₃, prepared with 2% and 3% iodized salt, volume slightly decreased due to high concentration of salt. Other external bread characteristics; crust colour, symmetry of form, character of crust and evenness of bake were non-significantly affected by different treatments. Internal bread characteristics (grain, aroma, texture and mastication of bread) are non-significantly affected by fortificants added except taste. The lowest score for taste of bread was found in T₃ (3% iodized salt). The score for taste of bread was gradually increased as the amount of KI used in bread was increased. T₆ prepared with 1.09 mg/100g KI had maximum score of taste.

**Fig. 1.** Sensory evaluation of bread

Conclusion

Bread fortified with iodized salt contained low level of iodine not enough to fulfill RDI but supports the projects working against IDD and its good practice to use iodized salt instead of non-iodized salt while KI fortified bread can alone achieve the goal to mitigate IDD. K content slightly increases in KI treatments. However, in sensory attributes only volume was affected in treatment in which 3% iodized salt was used, so volume of bread decreases if salt concentration is increased in bread than the recommended level. Addition of KI had positive effect on taste of bread. Stability test showed 15-20% iodine losts during processing of bread till the end of the process though it is the best means to get required iodine. Iodized salt alone would not be able to accomplish the objective but even just 2 slices of KI fortified bread provide RDI of iodine.

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