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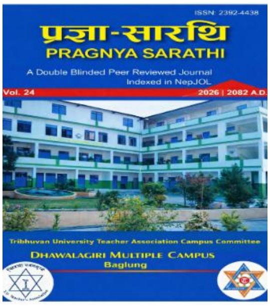

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Quantitative Determination of Iodine in Commercial Salt Samples

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Abstract

Iodine is an essential for the synthesis of thyroid hormones, and it has biological significance for tissue growth and development. Iodine deficiency disorder and the Universal iodization of table salt are widely recognized as effective strategies for its prevention. The specific objective of this research is to analyze the iodine content (in ppm) of commercially available salt samples collected from the Pokhara Valley, Nepal, using iodometric titration and a Rapid Test Kit (RTK). Iodine content in common salt used in the Pokhara valley should be ensured to ensure people's health. About 20-30 grams of 60 different salt samples were collected from the Pokhara Valley in plastic pouches. 10 different salt samples from varied batches were purchased, and one salt sample with batch SRC-09 was studied for its iodine content over 11 days. Both the RTK and the titration methods were used. A repeated 11 days' analysis of batch SRC-09 revealed a significantly higher iodine level than the reference value of 50 ppm ($t = 3.76$, $p < 0.05$), whereas batch wise analysis ($n = 10$) revealed a mean of 53.25 ± 16.89 ppm (range: 38.052–82.446 ppm) with no statistically significant difference between batches ($p > 0.05$). This consistency indicates that the manufacturing process and iodization were well controlled and uniform. The iodine content of common salt used in the Pokhara valley will help improve the quality of common salt from the authorized organization.

Keywords: iodized salt, iodine deficiency disorders, rapid test kits, iodine content analysis, Pokhara valley

Introduction

Iodine is an essential component of thyroid hormone synthesis and is crucial for tissue growth and development (Morreale de Escobar et al., 2004). Salts are substances formed upon neutralization of acids (Kilpatrick, 1935). The Ghana Standard Authority's regulation of 100 ppm during production and 50 ppm at retail may be excessively high, or it may be that the companies involved in salt iodination are not following the guidelines (Doku & Bortey, 2018). Severe iodine deficiency causes disorders that impact about 400 million individuals in Asia alone. These conditions include congenital abnormalities, stillbirths, and abortions; endemic cretinism, which is primarily characterized by mental impairment, deaf mutism, and spastic diplegia, with less severe neurological defects linked to fetal iodine deficiency; and impaired mental function in children and adults with goitre linked to subnormal thyroxine levels in the blood (Hetzel, 1983). The majority of European nations continue to have mild to moderate iodine deficiencies. Programs for iodine prophylaxis are already in place, and their cost is trivial when compared to the undeniable health benefits (Vitti et al., 2001).

Iodine levels ranged from 43.5 to 61.4 ppm, with a median of 53.9 ppm found in 98.1% of 2117 household salt samples from seven districts of Nepal. 67.2% of the samples had iodine levels between 45 and 75 parts per million. Giri, B. et al. (2022) analyzed that 0.9% of samples had insufficient iodine, 13.3% had suitable iodine, and 83.9% had more iodine than the WHO range.

Likewise, iodine is necessary for both humans and animals because it is required for the synthesis of thyroid hormones such as thyroxine (T_4) and triiodothyronine (T_3). Iodine deficiency causes goiter in adults and stunted growth in children (Fernández-Sánchez et al., 2003).

The median and mean (±sd) iodine content in newly opened top-of-the-can salt samples were 44.1 and 47.5 ± 18.5 mg/kg (n = 88, range 12.7–129 mg I/kg), while the geometric mean and standard deviation were 44.70 and 1.41. Of the 88 samples, 47 were below the USFDA-recommended iodine content threshold, and six exceeded it. Five samples taken from the same container at varying depths revealed a broad range of iodine content homogeneity, from 1.2× (8.3% CV) to 3.3× (49.3% CV). The iodine level varied between containers and brands. High-humidity storage results in a significant loss of iodine, whereas low or dry heat has no effect (Dasgupta et al., 2008).

The main goals of this study were to determine the iodine content (in parts per million) of commercially available iodized salt samples from the Salt Trading Corporation in Pokhara Valley using iodometric titration techniques and the Rapid Test Kit (RTK). It also sought to determine whether the observed iodine levels were consistent with recommendations to prevent iodine deficiency disorder (IDD). By comparing iodine levels across different salt production batches, the study also assessed batch-to-batch variability. The stability and consistency of the concentration of iodine analyzed in a specific batch (SRC-09) over an 11-day period of time by t-test.

Materials and Methods

Chemicals and Reagents

The chemicals and reagents used in this research were 0.005N sodium thiosulfate (anhydrous, extra pure analytical reagent, 99.5%), 0.1N potassium dichromate, 1% starch, and 10% potassium iodide (KI). Additionally, both dilute and concentrated HCl and H_2SO_4 are from the same supplier to maintain consistency. The apparatus used for the experimental work was a burette, a pipette, an electric oven, a 250ml conical flask, beakers, test tubes, a measuring cylinder, a grinder and pestle, plastic sample pouches, an electronic pH meter, and a rapid test kit.

Study Area

The salt samples used in this research were collected from the Pokhara Valley in Nepal. A total of 60 samples were taken. Each sample was placed in a separate, clean, air-tight plastic pouch to prevent contamination and iodine evaporation from the salt. Once the samples were collected and transported to the laboratory for research analysis. The analysis was conducted by measuring the pH, iodine content, and moisture content of each sample using standard chemical testing methods. The pH was measured with a pH meter, and the iodine content was measured using the oven-drying method. The batch number of 11 salt packets was noted. Also, one salt packet with batch SRC-09 was opened on 27th November 2022 at 8:43 am, and iodine percentage was calculated for 11 days.

Sampling Method and Justification of Inclusion/Exclusion Criteria

This research used a cross-sectional laboratory-based analytical design to measure the iodine content of commercially available iodized salt in Pokhara Valley. A major distributor of iodized salt in the Pokhara valley supplied the salt samples. A total of 60 sealed salt packets, representing different production batches, were selected at random to ensure representation of available stock. 20-30g of nine samples were collected and stored in sterile, sealed plastic pouches to prevent contamination and loss of iodine due to exposure, and transported to the chemistry laboratory at Prithvi Narayan Campus for analysis.

The data quality and reliability were ensured by establishing clear inclusion and exclusion criteria. The inclusion criteria were iodized salt packets available for retail distribution that were sealed and properly labeled; samples with a pH within the normal range of 6.5 to 8.5; and packets from different production batches.

By using these standards, only salt samples that met basic chemical quality standards and were ready for consumption were evaluated. The acceptable pH range (6.5 - 8.5) was selected because of the need to avoid extreme acidity or alkalinity and to ensure accurate titration. Salt samples having pH values below 6.5 or over 8.5, broken, unopened, or incorrectly stored packets, and samples with unclear batch identification were among the exclusion criteria. Because pH abnormalities can affect iodine stability and lead to imprecise estimation, samples outside the normal range were not included. Similar to this, to prevent skewed results due to environmental iodine loss, broken or incorrectly stored packets were excluded.

Iodometric titration and Rapid Test Kit (RTK) analysis were performed on the 53 samples remaining after 7 of the 60 samples were eliminated based on these criteria. Ten different production batches were also chosen for the examination of batch variance. One particular batch (SRC - 09) was observed for 11 consecutive days to evaluate the temporal consistency of iodine concentration and its stability. The validity, reliability, and scientific rigor of the study findings were improved by this methodical sampling strategy and well-reasoned inclusion and exclusion criteria.

Reagent Preparation and Standardization

1g of starch was weighed and dissolved in 100ml of distilled water to prepare a 1% starch solution, which was then heated to 80 °C for 10 minutes while stirring, then cooled and filtered before being stored. 0.624g of $Na_2S_2O_3.5H_2O$ was taken and dissolved in distilled water and diluted to 1000ml to prepare a 0.005N sodium thosulphate solution. Then standardized using the primary standard K_2Cr_2O in an acidic medium with a starch indicator, yielding a final concentration of 0.0052N. Further, 10 g of KI was dissolved in 100ml of distilled water to prepare a 10% KI solution, and 26.63ml of 98% concentrated acid (sp. gr. 1.84). Finally, it was then diluted with distilled water to the necessary amount to create 2N H_2SO_4 (250ml)

Determination of iodine content by Iodometric Titration

5g of the salt sample was weighed and poured into a clean Erlenmeyer flask, and 25 ml of distilled water was poured to make a solution. Then added 5ml of 10% KI solution and 2-3 ml of 2N H2SO4 with swirling. The iodine starch complex changes into yellow when sodium thiosulphate (Na2S2O3) which was added to the mixture. After that, added 1% starch to the solution and titrated it. Lastly, the amount of (Na2S2O3) was recorded (Kleks & Davidson, 1966).

Calculation

The weight of salt sample (W) = 5 gm
Normality of $Na_2S_2O_3$ (N) = 0.005N
Volume of $Na_2S_2O_3$ used in titration (V) = x (say for now)

Factor = 2.114×1000=21140 (converting gram to milligrams)
Iodine content = $\frac{V \times N \times 21140}{W}$ ppm
(Kleks & Davidson 1966)

The Merck Biosciences India (MBI) RTK (Rapid Iodine Test Kit)

A color shift was observed when the two reagents in the kit were added dropwise to the salt. The strip is included in the kit along with color charts for 0 ppm, below 15 ppm, and above 15 ppm. The iodine concentration in the salt solution can be determined using the color chart.

Data Collection and Analysis

Salt samples were collected from various sources as part of the data collection method used in this study to calculate the iodine content of salt in the Pokhara Valley. Salt samples were taken from Pokhara Nepal for the research. The research area was used to calculate primary data. To guarantee a variety of samples that could be utilized for the study, the samples were gathered over a 15 days' period.

The research study on the iodine level of salt in Pokhara was used statistical analysis through t - tests, histograms, line graphs, and bar graphs. The t-test was employed to assess whether there was a significant difference in iodine concentration among salt samples collected on different days. For the purpose of statistical analysis SPSS software was used.

Iodometric Titration of Iodized Salt

Five grams of the salt sample was dissolved with 25 mL of distilled water in a clean Erlenmeyer flask and added 5 mL of 10% KI solution. And also 2 - 3 ml of 2N H_2SO_4 was also poured to the flask and swirled to make mixture. The mixture was titrated with 0.005 M sodium thiosulfate ($Na_2S_2O_3$) until disappearance of the yellow color of the iodine-starch complex. The solution was added with 1ml of 1% starch and titrated until disappearance of blue color. Lastly, the volume of $Na_2S_2O_3$ was recorded (Kleks & Davidson, 1966).

Calculation

The weight of salt sample (W) = 5 gm
Normality of sodium thiosulphate = 0.005N
Volume of sodium thiosulphatein titration (V) = x (say)

Factor = 2.114×1000=21140 (converting gram to milligrams)
Iodine content = (V×N×21140)/W ppm
(Kleks, & Davidson, 1966)

Results and Discussion

Sixty salt samples were collected from Pokhara Valley for the analysis of iodine percentage. Of the 60 samples, 7 were excluded from the analysis because they did not meet the inclusion criteria. The remaining 53 samples were analyzed using the titration method. The data obtained from the analysis are presented in Table 1. The iodine percentage in the salt samples ranged from 23.254 ppm to 135.296 ppm, with a mean value of 53.798 ppm and a standard deviation of 22.907 ppm. The result of this research of salt sample is a significant variation in the iodine percentage among the different batches of salt sample. The iodine percentage ranges from 38.052 ppm to 82.446 ppm, with an average of 53.25 ppm. The standard deviation of data of this research is of 16.89 ppm. The highest iodine percentage was found in batch TFL-03 (82.446 ppm) while the lowest was found in batch ACL-99 (38.052ppm).

Table 1

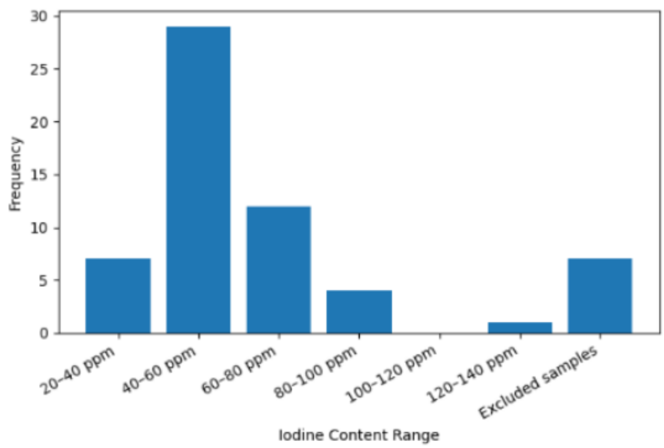
Iodine Content in Salt Samples (in ppm)

Salt Sample	Iodine Content (ppm)	Salt Sample	Iodine Content (ppm)	Salt Sample	Iodine Content (ppm)
S1	35.938	S19	67.648	S37	52.850
S2	40.166	S20	67.648	S38	52.850
S3	65.534	S21	88.788	S39	50.736
S4	40.166	S22	46.508	S40	48.622
S5	40.166	S23	35.938	S41	38.052
S6	135.296	S24	59.192	S42	46.508
S7	35.938	S25	52.850	S43	42.280
S8	71.876	S26	59.192	S44	42.280
S9	65.534	S27	78.218	S45	52.850
S10	65.534	S28	57.078	S46	54.964
S11	57.078	S29	46.508	S47	44.394
S12	80.332	S30	54.964	S48	38.052
S13	67.648	S31	86.674	S49	78.218
S14	71.876	S32	61.306	S50	57.078
S15	54.964	S33	57.078	S51	82.446
S16	25.368	S34	63.420	S52	46.508
S17	23.254	S35	40.166		
S18	46.508	S36	57.078		

Note. Iodine content is reported in parts per million (ppm).

Figure 1

Distribution of Iodine Content in Salt Samples



The iodine percentage of Salt with batch SRC - 09 was analyzed for 11 days using the MBI iodine test kit as well as titration. The iodine content of the salt samples ranged from 46.508 ppm to 54.964 ppm. The data were analyzed, and the mean iodine percentage for batch SRC-09 was 52.01 ppm, with a standard deviation of 2.36 ppm.

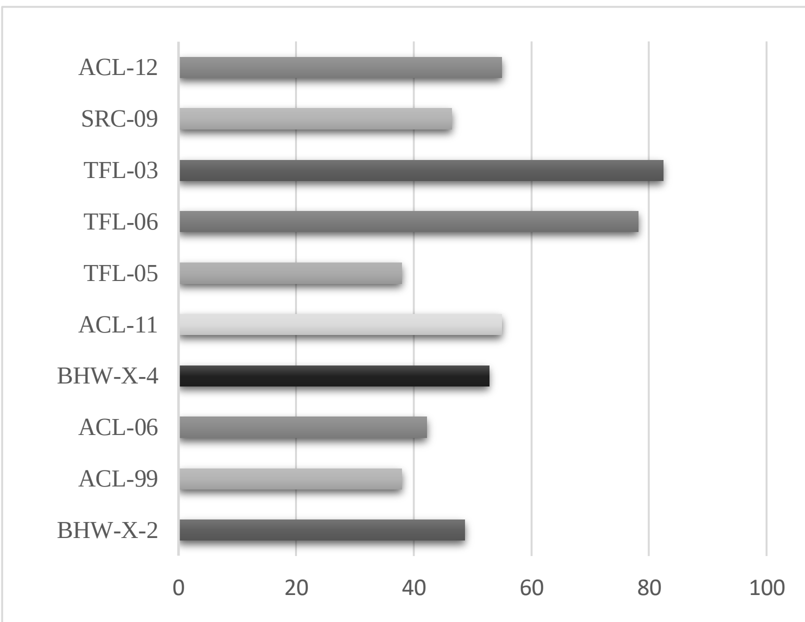
Table 2

Table Showing Iodine Content in Different Batches of Salt

Samples	Batch of collected samples	Iodine percentage (in ppm)
SA1	BHW-X-2	48.622
SA2	ACL-99	38.052
SA3	ACL-06	42.28
SA4	BHW-X-4	52.85
SA5	ACL-11	54.964
SA6	TFL-05	38.052
SA7	TFL-06	78.218
SA8	TFL-03	82.446
SA9	SRC-09	46.508
SA10	ACL-12	54.964

Figure 2

Bar Diagram of Iodine Content in Different Batches of Salt



Analysis of Iodine Content in Salt Samples of Batch SRC-09

The iodine percentage of Salt with batch SRC-09 was analyzed for 11 days using the MBI iodine test kit and by titration. The iodine content of the salt samples ranged from 46.508 ppm to 54.964 ppm. The data were analyzed, and the mean iodine percentage for batch SRC-09 was 52.01 ppm, with a standard deviation of 2.36 ppm.

Hypothesis Testing of Iodine Content in Salt (Batch SRC-09)

The present analysis examined whether the mean iodine content of the salt samples (Batch SRC-09) differs significantly from the standard value of 50 ppm. A one-sample *t*-test was used to test the null hypothesis that the population mean iodine content equals 50 ppm. The sample mean iodine content was 52.47 ppm, with a standard deviation of 2.174, based on 11 observations.

Table 3

Variation of Iodine Content in Salt Samples of Batch SRC-09 Over 11 Days

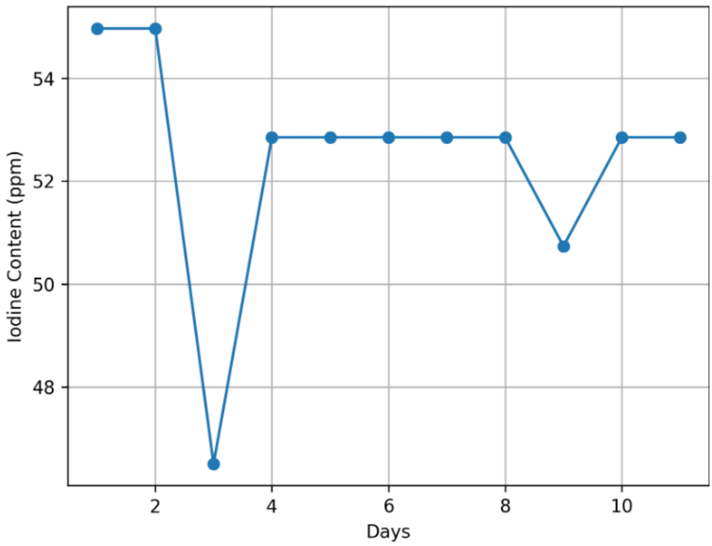
Samples	Batch	Iodine Content (ppm)
Day 1	SRC-09	54.964
Day 2	SRC-09	54.964
Day 3	SRC-09	46.508
Day 4	SRC-09	52.850
Day 5	SRC-09	52.850
Day 6	SRC-09	52.850
Day 7	SRC-09	52.850
Day 8	SRC-09	52.850
Day 9	SRC-09	50.736
Day 10	SRC-09	52.850
Day 11	SRC-09	52.850

Note. ppm = parts per million.

The computed test statistic was $t_{(10)} = 3.76$ at a significance level of $\alpha = 0.05$. For a one-tailed test with 10 degrees of freedom, the critical *t*-value is ±1.812, depending on the direction of the test. Since the research objective is to determine whether the iodine content exceeds 50 ppm, a right-tailed test is appropriate. Thus, the critical value is +1.812. Because the calculated *t*-value (3.76) exceeds the critical value (1.812), the result falls within the rejection region. Therefore, the null hypothesis is rejected.

Figure 3

Analysis of Iodine Content of Salt Samples Across 10 Batches



A statistical analysis was conducted to assess whether there is a significant difference in iodine content across 10 salt batches. The null hypothesis (H_0) stated that there is no significant difference in the mean iodine content between the batches, whereas the alternative hypothesis (H_1) stated that a significant difference exists. The mean iodine content of the samples was $M = 53.70$ ppm, with a standard deviation of $SD = 14.59$ ppm. The iodine values ranged from 38.052 ppm to 82.446 ppm, indicating substantial variability across batches. A one-sample *t*-test was performed with an assumed population mean equal to the sample mean ($\mu = 53.70$ ppm), yielding $t(9) = 0.00$ at the 5% significance level. Since the calculated *t*-value was less than the critical value (±2.262), the null hypothesis was retained. However, this result should be interpreted with caution. The use of a one-sample *t*-test in this context is not appropriate for comparing multiple batches, as the test is designed to compare a sample mean with a known or hypothesized population mean. Furthermore, assuming μ equals the sample mean inherently yields a t-value of zero, rendering the test statistically uninformative. Therefore, although the statistical test suggests no significant difference, this conclusion is not methodologically valid. Descriptive statistics indicate noticeable variation in iodine content among the batches. A more appropriate method, such as one-way analysis of variance (ANOVA), would be required to properly assess differences among multiple batches.

Conclusion

The analyzed research data shows considerable differences in iodine concentration among the salt samples. The iodine levels in salt are crucial for preventing conditions linked to iodine deficiency which continue to be prevalent worldwide. The iodine concentrations in salt samples from different batches and areas vary considerably.

The iodine concentrations in salt samples collected from the Pokhara valley ranged from 23.252 ppm to 135.296 ppm, with an average of 55.123 ppm. The results of this research show that certain samples contain low iodine levels, which pose iodine deficiency issues for individual consumers.

The second dataset comprises 10 batches of salt, with iodine concentrations ranging from 38.052 ppm to 82.446 ppm. This result shows significant variation in iodine concentrations across different salt batches, underscoring the importance of regularly checking iodine levels in salt.

The third dataset focused on a specific batch of salt (SRC-09) and showed that the iodine content remained relatively consistent over 11 days of testing, with an average iodine content of 52.857 ppm. The results of the research findings are crucial for salt manufacturers and authorities, who recognize the importance of maintaining a stable iodine concentration in salt over time.

In conclusion, the findings of the research emphasize the necessity of regular monitoring of iodine levels in salt to ensure that consumers receive adequate iodine and prevent iodine deficiency problems. This result also underscores the importance of using appropriate testing methods to accurately assess iodine levels in salt.

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Consent for publication: Not applicable

Conflict of interest: The author does not have any conflict of interest with any institutions concerning this research

Ethical conduct of research: This paper is written ethically

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