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Research Article

Assessment of Nickel Contamination in Vanaspati Ghee from Hyderabad Pakistan and its Food Safety Implication

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ABSTRACT

Vanaspati ghee is widely consumed in many South Asian countries. It is produced through the partial hydrogenation of vegetable oil using nickel catalysts. Despite many purification steps, residual nickel remains in the vanaspati ghee and poses health hazards. This study aimed to determine the nickel in nine commercially available vanaspati ghee samples collected from Hyderabad, Sindh, Pakistan and to compare against the PSQCA recommended limit. Nickel in the ghee was determined by using flame atomic absorption spectroscopy (FAAS) after the wet acid digestion. The method was found to be linear (R2=0.9997) with a limit of detection (0.008 µg/g) and limit of quantification (0.025 µg/g). Results showed that branded commercial ghee contained the nickel in the range of 0.075–0.168 µg/g while locally produced and blended ghee contained nickel in the range of 0.167–0.651 µg/g. One blended sample marginally exceeded the PSQCA recommended limit of 0.2 µg/g. These findings highlight the need for regulatory monitoring of residual nickel in vanaspati ghee.

Keywords: Nickel; Vanaspati ghee; Flame atomic absorption spectrometry; Food safety.

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INTRODUCTION

Vanaspati ghee is a partially hydrogenated vegetable oil that is used as a cheap cooking fat in South Asia (Sami et al., 2023). As a cheap alternative to desi ghee (clarified butterfat), vanaspati ghee constitutes nearly 40–50% of the total edible fat consumption in Pakistan (Saha et al., 2023; Tahir et al., 2013; Xia et al., 2021). Vanaspati ghee is produced through the catalytic hydrogenation of liquid vegetable oils (palm oil, cottonseed oil, soybean oil, and their blends) to convert them into saturated fat in the presence of Nickel metal catalyst (Codex Alimentarius Commission, 2019). Despite many post-production filtration steps, residual nickel remains, in the fat which may create serious health implications for the consumers (Achaya, 2018; Ansari et al., 1994; Khan et al., 2007).

Nickel is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2012) and its chronic exposure can cause various health effects. Nickel ions (Ni^{2+}) disturb vital metabolic processes at the cellular level by displacing zinc, calcium, and magnesium from their corresponding binding sites on regulatory proteins and enzymes thereby disturb the homeostasis (Genchi et al., 2020). Reactive oxygen species (ROS) produced by nickel have been shown to cause oxidative stress, which in turn causes lipid peroxidation, DNA strand breaks, and protein oxidation, all of which increase genotoxicity and carcinogenicity (Costa, 2023). Additionally, long-term dietary exposure to nickel has been linked to immunotoxicity, nephrotoxicity, reproductive toxicity, and contact dermatitis (Ahlström et al., 2019)

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The European Food Safety Authority (EFSA) has established a tolerable daily intake (TDI) of 2.8 µg nickel per kilogram of body weight per day (Chain et al., 2020), while the World Health Organization (WHO) has set a provisional tolerable daily intake (PTDI) of 70 µg per person per day (Organization, 2021). The Pakistan Standards and Quality Control Authority (PSQCA) oversee the country's food safety regulations regarding heavy metals in edible oils and fats. PSQCA guidelines for vanaspati ghee (PS: 221-2023, 5th Revision) has set the permissible limit for nickel at 0.2 mg/kg in the final product (PSQCA, 2023). In consistent with PSQCA, the Punjab Food Authority (PFA) imposes maximum residual limits of 0.2 mg/kg for nickel in vanaspati ghee (PFA, 2018).

Given the widespread and daily consumption of vanaspati ghee in Pakistan due to its lower cost, the studies on the presence of nickel in the final product are scarce. Therefore, this study aimed to determine the residual nickel in vanaspati ghee from different commercially available samples in Hyderabad, Sindh, Pakistan and to assess the health implications in the context of the PSQCA guidelines.

MATERIALS AND METHODS

Sample Collection

Nine vanaspati ghee products (branded, locally produced, blended) were purchased from three retail markets in Hyderabad, Sindh, Pakistan between September and November 2023. Selection criteria included market availability, category representation, brand popularity, manufacturing origin, and label integrity. Damaged or uncertain-origin products were excluded. Four samples were commercially packaged branded vanaspati ghee products from major national manufacturers (coded VS-1 to VS-4), three were locally produced vanaspati ghee from small-scale regional manufacturers (VS-5 to VS-7), and two were blended vanaspati ghee products commonly used by bakeries and commercial food establishments (VS-8, VS-9). All samples were collected in triplicate in acid-washed amber glass containers to prevent contamination and photodegradation. Sample labels, manufacturing dates, and batch numbers were recorded for each product.

Reagents and Standards

All reagents and chemicals used were of analytical grade. Concentrated nitric and Perchloric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure deionized water was produced using a Milli-Q (Merck Millipore, Billerica, MA, USA). A certified single-element nickel standard solution (1000 µg/mL in 2% HNO_3) was obtained from Fluka Analytical (Buchs, Switzerland) for the preparation of calibration standards.

Sample Digestion

The wet acid digestion procedure was adapted from AOAC Official Method 999.11 with minor modifications optimized for the fatty matrix of vanaspati ghee (Horwitz & Latimer, 2005). Briefly, 2.0 g of each vanaspati ghee sample was accurately weighed into a 100 mL borosilicate digestion tube, and mixture of 10 mL concentrated HNO_3 and 2 mL $HClO_4$ was added to it. The digestion tubes were placed on a hotplate and the temperature was progressively raised: initially maintained at 60°C for 30 minutes to allow gentle initial oxidation, then increased to 120°C for 45 minutes, and finally to 180°C until the digest became clear and colorless. The digests were quantitatively transferred to 25 mL volumetric flasks and diluted to volume with 1% (v/v) HNO3 after cooling to room temperature.

Instrumentation and Operating Conditions

Nickel determination was carried out using Shimadzu AA atomic absorption spectrometer equipped with nickel hollow cathode lamp. The instrument was operated under the following optimized conditions: wavelength, 232.0 nm with lamp current, 25 mA; spectral slit width, 0.2 nm. Calibration standards were prepared by appropriate serial dilution of the 1000 µg/mL nickel stock solution in 1% HNO_3 to yield concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 µg/mL

Method Validation

The analytical method was validated in-house following the guidelines outlined in ICH Q2(R1) (ICH, 2005) and AOAC guidelines for single-laboratory validation. The parameters that were validated were Linearity, by analyzing six calibration standards (0.5–5.0 µg/mL); limit of detection (LOD) and limit of quantification (LOQ) by using the signal to noise ratio method. Accuracy was assessed through recovery experiments by spiking a blank matrix sample (previously confirmed nickel-free homogenized ghee sample) with standard solutions at three concentration levels (0.05, 0.10, and 0.20 µg/g). Spiked samples were allowed to equilibrate for 30 minutes at room temperature before extraction, and followed the same procedure as that of actual samples. Recovery was calculated by comparing the measured concentration in spiked samples to the known added concentration and expressed as a percentage. Precision was determined and expressed as relative standard deviation (%RSD) of triplicate measurements for each sample.

Statistical Analysis

Analysis was done in triplicate and results are reported as mean ± standard deviation. Statistical analysis was carried out by using the IBM SPSS version 26. The Shapiro-Wilk test ($p > 0.05$) was used to evaluate the assumptions of normality and homogeneity of variances prior to One-Way ANOVA, and Levene's test ($p > 0.05$) was used to confirm homogeneity of variances. One way ANOVA was used to determine the statistical significance in amount of the nickel concentration determined in three categories (branded, local, and blended).

RESULTS

Calibration and Method Validation

The calibration curve for nickel determination was constructed using six standard solutions in the concentration range of 0.5–5.0 µg/mL (Figure 1). The linear correlations with $R^2 = 0.9997$ was achieved indicating a strong linear relationship between absorbance and nickel concentration. Figure 1 presents the calibration curve with the regression equation $y = 0.0624x + 0.0007$, where y represents the absorbance and x represents the nickel concentration (µg/mL).

The method performance characteristics were determined and presented in table 1. the limit of detection and limit of quantification were found to be 0.008 µg/g, 0.025 µg/g respectively (Table 1). Accuracy was determined by assessing the recovery and found in the range of 97.4–102.6%, which is acceptable according to ICH guidelines (ICH, 2005). Repeatability of the results were assessed by measuring the relative standard deviation (%RSD) and found in the range of 1.8% to 4.2% in all samples which is well within the ≤5% accepted criteria(ICH, 2005).

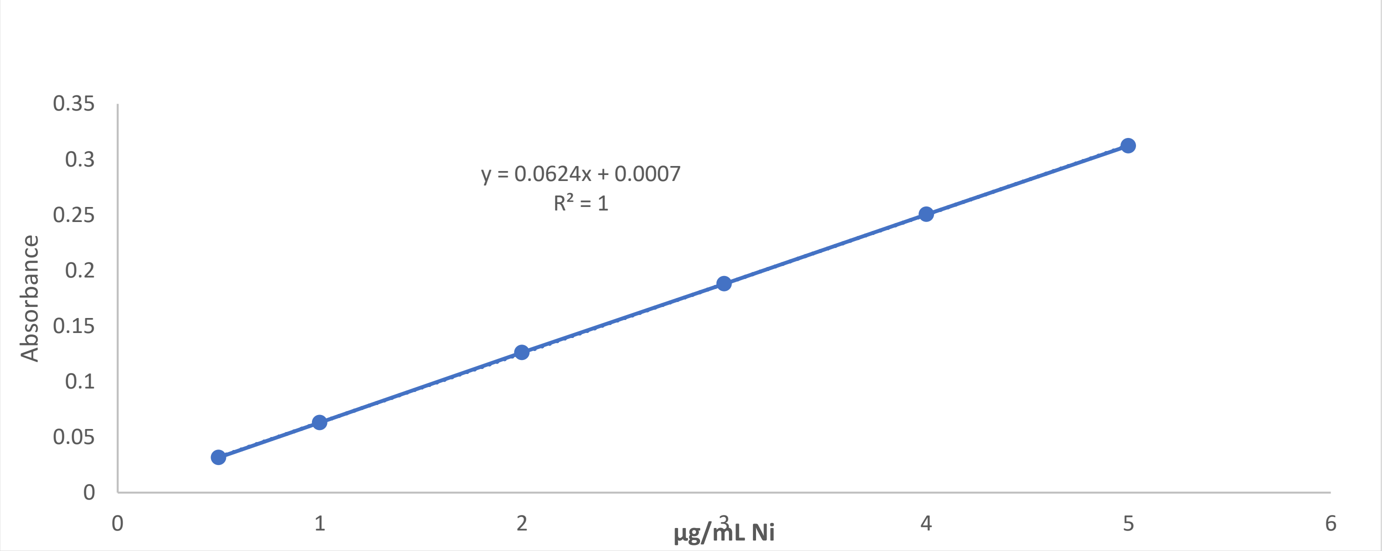

Figure 1. Calibration curve for nickel determination by FAAS.

Table 1. Method Validation Parameters for Nickel Determination.

Parameter	Observed Value	Acceptance Criterion
Linearity (R^2)	0.9997	≥ 0.999
LOD (µg/g)	0.008	< 0.05
LOQ (µg/g)	0.025	< 0.10
Accuracy – Recovery (%)	97.4–102.6	90–110%
Precision – RSD (%)	1.8–4.2	≤ 5%
Wavelength (nm)	232.0	Ni AAS standard
Flame type	Air–Acetylene	Standard

Nickel Concentrations in Vanaspati Ghee Samples

The concentration of nickel determined in samples collected from Hyderabad is presented in Table 2. The Mean concentration of nickel was found in the range of 0.075 µg/g in VS-3 (Brand C) to 0.651 µg/g in VS-9 (Blended-2). A relatively higher concentration was detected in the blended sample. The branded commercial samples had nickel concentrations much lower, and in the range of 0.075 to 0.168 µg/g, well below PSQCA guidelines of 0.2 µg/g. However, the locally produced vanaspati ghee samples (VS-5 to VS-7) contained intermediate nickel levels in the range of 0.167 to 0.199 µg/g. Blended vanaspati ghee products (VS-8 and VS-9) exhibited relatively higher nickel concentrations of 0.200 and 0.651 µg/g, respectively.

Table 2. Nickel concentrations in vanaspati ghee samples.

Sample ID	Mean ± SD (µg/g)
VS-1 (Brand A)	0.088 ± 0.004
VS-2 (Brand B)	0.142 ± 0.005
VS-3 (Brand C)	0.075 ± 0.003
VS-4 (Brand D)	0.168 ± 0.007
VS-5 (Local-1)	0.181 ± 0.008
VS-6 (Local-2)	0.199 ± 0.007
VS-7 (Local-3)	0.167 ± 0.009
VS-8 (Blended-1)	0.200 ± 0.012
VS-9 (Blended-2)	0.651 ± 0.013
PSQCA	>0.2

Values expressed as mean ± SD. RSD = relative standard deviation.

One-way ANOVA confirmed the significance difference among the different found in collected categories analyzed for the Nickel concentration. One blended vanaspati ghee samples (VS-9: 0.651 µg/g) exceeded PSQCA maximum permissible nickel limit of 0.2 µg/g in Vanaspati ghee under PS:221-2003, reflecting the residual catalyst threshold deemed safe following hydrogenation processing.

DISCUSSION

The primary source of nickel in the ghee products is insufficient removal of nickel being used as catalyst (Meshram & Pandey, 2019). During the catalytic hydrogenation, it is used in the range of 0.1–0.5% w/w of the oil. Sometime higher catalyst is used to achieved efficient hydrogenation process, leaves higher concentration of the nickel in ghee products (Begum et al., 2022). The most commonly used methods for the removal of catalyst include are, hot filtration through bleaching earth, activated carbon adsorption, and steam deodorization (List, 2009). The branded manufacturers have advance equipment and methods to remove the nickel, however small-scale vanaspati ghee producers frequently rely on single-stage filtration without subsequent activated carbon treatment which may lead to substantially higher residual nickel in the finished product (Abd Hamid et al., 2021)

The results obtained in the present study are consistent with published data on nickel contamination in vanaspati ghee from Pakistan. Khalid et al. reported nickel concentrations of 0.1–3.98 µg/g in 12 vanaspati ghee samples from Punjab (Khalid, 1991),broadly comparable to the range observed in the present study. However, the upper end is notably higher than our findings, which may reflect improvements in industrial filtration practices over the past three decades or differences in the brands sampled. Ansari et al reported nickel levels of 0.1-3.5 µg/g yielding a mean of 0.31 µg/g (Ansari et al., 1994). The value is consistent with the lower range of our findings, suggesting that nickel contamination has persisted at similar baseline levels despite the passage of time. Yaqub et al reported nickel concentration of 0.395-1.234 mg 100 g (Yaquband et al., 1999). When converted to comparable units (3.95–12.34 µg/g), these values are considerably higher than those recorded in the present study, which may be attributable to differences in sampling regions and production methods. Bashir et al. (2017) reported a mean nickel concentration of 1.01 µg/g in hydrogenated ghee fractions (Bashir et al., 2017), which is higher than the mean value recorded in the present study. Khan et al. (2007) recorded nickel concentrations ranging from 0.05 to 0.63 µg/g with a mean of 0.31 µg/g, noting that some samples surpassed PSQCA thresholds (Khan et al., 2007). In comparison, the present study recorded 0.207 µg/g, which is lower to the mean reported by Khan et al., suggesting that nickel contamination levels in Pakistani vanaspati ghee have decreased over the intervening period. The fact that both studies identified samples exceeding PSQCA limits underscores the persistence of public health concern

Rehman (2015) found higher contamination levels in the Khyber Pakhtunkhwa (KPK) region, with nickel ranging from 0.08 to 1.22 µg/g and a mean of 0.54 µg/g, exceeding the PSQCA limit (Rehman, 2015). The values recorded in the present study from 0.207 µg/g are lower to those reported by Rehman (2015) from KPK, indicating that geographical variation in nickel contamination exists across Pakistani provinces. The persistently elevated nickel levels across multiple Pakistani studies spanning over three decades from Khalid (1991) to the present study collectively indicate that voluntary compliance with good manufacturing practices has been insufficient and needs continuous monitoring.

A health risk assessment was conducted using EFSA's tolerable daily intake (TDI) of 2.8 µg/kg body weight/day for nickel. For an average Pakistani adult (65 kg), this equals 182 µg/day. At typical vanaspati ghee consumption of 25 g/day (NNS, 2018), the most contaminated sample (VS-9: 0.651 µg/g) contributed 16.3 µg/day (8.9% TDI), while branded ghee (VS-3: 0.075 µg/g) contributed only 1.9 µg/day (1.0% TDI). Although vanaspati ghee alone may not exceed TDI, cumulative exposure from other nickel-rich foods legumes, grains, and vegetables raises concern, particularly for high-consumption groups(Khalid, 2025).

Collectively, these findings highlight the need for regular monitoring of nickel in vanaspati ghee, with particular emphasis on small-scale and unbranded producers PSQCA consistently appear to demonstrate poorer compliance with safe nickel residue levels, posing potential health risks to local populations who rely heavily on vanaspati ghee as a primary cooking fat.

CONCLUSION

This study reported the residual nickel contamination in the vanaspati ghee by using flame atomic absorption spectrometry (FAAS) from Hyderabad, Sindh, Pakistan. Nickel had been detected in the range of 0.075 to 0.651 µg/g, and there were significant variations between product categories (one-way ANOVA, $p < 0.001$). The lowest concentration of nickel was found in branded products (0.075–0.168 µg/g), followed by locally produced ghee (0.167–0.199 µg/g) and blended vanaspati ghee (0.200–0.651 µg/g). These results suggest that manufacturing scale and product type are important factors in determining the levels of nickel residues.

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AUTHOR CONTRIBUTIONS

Zulqar Nain: Data collection, statistical analysis and manuscript drafting. Zaheer Ahmed: Provided seed material. Ahmed Raza: Technical support, sowing planning, field management. Muhammad Qasim, Maqsood Ali, Uzair Ahmad, Faiz Ullah Arif, Muhammad Nasir: Field management and data collection. Muhammad Rizwan Shafiq: Data verification, manuscript review. Sidra Iqbal: Conceptualization, study supervision, research design, critical revision of manuscript, final approval of version for submission. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

COMPETING OF INTEREST

The authors declare no competing interests.

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