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Physico-chemical Analysis and Metallic Contamination of Reused Oil and Savory Snacks Available at University Canteens

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ABSTRACT

Edible oils play a crucial role in our daily diet, providing energy, essential fatty acids, and vitamins. Frying is a common method for preparing many foods, but the deep-frying process can alter the composition of both oil and food. **Objectives:** To assess the potential health risks associated with the consumption of reused oil and savory snacks. **Methods:** A total of 25 oil samples, including Before Frying Oil (BFO) and After Frying Oil (AFO), as well as snack samples (samosa and pakora), were collected from the canteens of Sindh University, Jamshoro, Pakistan. Blood samples were collected from students studying at the main campus of Sindh University, Jamshoro, and control samples were also collected from individuals of the same age and gender. Elements like copper (Cu), iron (Fe), nickel (Ni), lead (Pb), zinc (Zn) and chromium (Cr) were analyzed from oil, blood and snacks samples by atomic absorption spectrometry. **Results:** Metals like Cu, Fe, Ni, Pb, Cr increased along with Zn decreased in AFO samples whereas all metals like Cu, Fe, Ni, Pb, and Cr were found increased and Zn decreased in blood of consumers, Cu, Fe, Ni, Pb, Cr decreased in Samosa and Zn increased in Savory samples. **Conclusions:** Used cooking oil is dangerous for human health. People should avoid buying contaminated food items, especially savory snacks, from open markets because they are harmful to health.

INTRODUCTION

Nowadays, the deep-fried snacks are being used at home, restaurants and workplaces, which mostly use canola oil for frying purposes. Many Asian countries prepare snacks in different ways; moreover, in local restaurants, shops and vendors do not observe proper hygiene and use oil for frying many times. Students don't have breakfast at home or hostel and use savory snacks [1]. South Asian countries, especially Pakistan, India, and Bangladesh, are developing countries, where University students belong to the middle socio-economic class, so they have less pocket money. Canteens of universities prepare savory snack items (samosa, pakora, roll, etc.) at very low prices, which are fried repeatedly in frying oil [2]. In different developing countries, especially Asian countries like Pakistan, India, China, and Thailand, shop vendors prepare snacks. These are ready-to-eat snack foods at low cost in university canteens. In Pakistan, generally, people use street vendor foods specially snacks daily. Street foods are prepared and even sold by local vendors at local markets, schools, parking lots, and universities, for direct consumption without further processing [3, 4]. In humans, ingestion of food contaminated with trace metals is the most common route of heavy metal (HM) exposure and can pose a serious health risk. Snacks are being used daily at schools and universities by students. Snacks' material or ingredients quality is usually not checked in local shops/Canteens.

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Moreover, the same oil is used many times by food vendors during the preparation of savory snacks [5]. These savory snacks are prepared in contaminated utensils, without poor sanitation, improper hygiene, improper cookware, inadequate storage conditions, and contaminated ingredients. High intake of snacks increases the risk of diseases; unhealthy eating habits lead to uncontrolled weight gain. Cooking oil deterioration is accelerated by the formation of degradation compounds such as free fatty acids and peroxides [6, 7]. The presence of trace metals in cooking oils depends on many factors: they might originate from the soil, fertilizers, plantations, presence of industry [8]. Metals may also be introduced during the contamination process through contact with packaging materials. The trace metals enhance the rate of oxidation of cooking oils by increasing the generation of free radicals from fatty acid hydro-peroxides [9, 10].

Despite the widespread consumption of deep-fried snacks by university students, limited studies in Pakistan have systematically assessed the extent of metallic contamination in reused cooking oils and associated health risks in consumers. Most previous research focuses on either oil quality or street food safety separately, leaving a gap in understanding the direct correlation between reused frying oil, toxic metal accumulation in snacks, and their impact on student health. This study addresses this gap by analyzing both physicochemical changes in oils and metal contamination in snacks and consumer blood samples. Metals were analyzed in 25 cooking oil samples (before and after frying), samosa and pakora samples collected from university canteens, and in blood serum from 30 student consumers and 30 non-consumer controls, to assess the potential health risks associated with the consumption of reused oil and savory snacks. This study aimed to determine the quality of oil and snacks used at University Canteens by the physicochemical properties and metals analysis.

METHODS

This cross-sectional study involved analysis of 25 cooking oil samples from the students' canteen of Sindh University, Jamshoro, Sindh, Pakistan, from May 2019 to July 2021. The study was started after ethical approval from the ethical committee at the Institute of Biochemistry, University of Sindh (Jamshoro) with a reference number IOB/48/2019. Informed consent was obtained from all students prior to the study. The sample sizes (25 oil samples, 30 consumers, and 30 non-consumers) were determined based on preliminary surveys of typical canteen operations and student consumption patterns. These numbers provided sufficient representation, while remaining logistically feasible, and consistent with similar studies on food contamination. The before/after frying comparison and consumer/non-consumer matching were designed to enhance the detection of meaningful differences. Samples were collected using purposive sampling. Twenty-five paired oil samples (before and after frying) and corresponding samosa and pakora samples were obtained from canteens that prepared and sold fried snacks on-site using unbranded oil. Canteens using branded or single-use oil were excluded. Blood samples were taken from 30 regular snack consumers and 30 matched non-consumers. A validated questionnaire (Cronbach's alpha=0.75) was filled out by students, consisting of questions about the habits of eating savory snacks. During the study, it was surveyed that all were using unbranded rapeseed cooking oil and the same cooking oil was used many times for frying snacks. Data were analyzed using SPSS-25.0. Metal concentrations and physicochemical parameters were compared using paired t-tests for before vs. after frying oil samples (BFO/AFO) and independent t-tests for consumer vs. non-consumer blood samples. Questionnaire data were analyzed using descriptive statistics and chi-square tests where appropriate. Normality was verified using Shapiro-Wilk tests; non-parametric alternatives (Mann-Whitney U, Wilcoxon signed-rank) have been used when assumptions were violated. Results with p-values <0.05 were considered statistically significant. Before studying, a survey was done for the collection of information from different canteens of the University for snack selection, mostly Samosa and Pakora were seen to be sold to students. Questionnaire responses were scored and analyzed using descriptive statistics, while metal concentration data were compared using independent t-tests with $p<0.05$ considered significant." Ultrapure water (Bucks, UK), Nitric acid (Merck, Darmstadt, Germany), Hydrogen peroxide (Merck), standards of Cu, Ni, Fe, Pb, Zn and Cr mg/L (Fluka-Kamica-Buchs, Switzerland). Glassware and plastic material were treated with 5M pure Nitric acid for 24 Hours. Nitric acid (65%) and Hydrogen Peroxide 30% (2:1 ratio) was used for CDM (Conventional digestion method). Standard solutions of Cu, Ni and Fe of 1000 mg/L were prepared by dilution of certified Standard solutions. Physicochemical Properties of Before Frying Oil (BFO) and After Frying Oil (AFO): Physical and chemical properties of before and after frying used oil were determined using already reported methods [11-14]. 5cc Blood samples were collected from 30 consumers and 30 non-consumer students, who were studying in different BS programs. The age range of students was from 17 to 25 years. Whole blood samples were collected and centrifuged at 4 °C for serum collection. Serum was used for the determination of toxic metals, i.e. Copper (Cu), Iron (Fe), Nickel (Ni), Lead (Pb), Zinc (Zn) and Chromium (Cr). Each composite sample of snack was mixed and ground in a mortar and pestle, then kept at -4 Cº. Weighed 1.0 g of each mix composite sample of samosa and pakora samples (electronic balance machine, A and D

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company C0006). Then kept the samosa and pakora samples for ashing in a furnace. Ash content was measured as an indicator of total mineral residue, serving as a proxy for potential metal accumulation in snacks. 600°C, for 2 hours. Afterwards, the snacks were re-weighed [15]. Ash content was measured as an indicator of total mineral residue, serving as a proxy for potential metal accumulation in snacks. This study correctly weighed triplicate samples (0.5 g) of each reused cooking oil, snack samples (samosa, pakora) and blood samples into individual (100 ml) conical flasks. Two (2) mL of concentrated HNO_3 (65%) and H_2O_2 (30%) (2:1, v/v; analytical grade, Merck) were added to each flask, incubated for 10 min at room temperature, and heated at 80 °C until clear. Reagent blanks were run to validate digestion and control for background contamination. After evaporation of flask contents, the semi-dried mass was allowed to dissolve in 10 ml 0.2 M HNO3, and it was then filtered through filter paper (Whatman No. 42) and a final volume was made up to 10 ml in volumetric flasks with ultrapure water, which was designated as the stock solution. All samples were prepared in triplicate for reproducibility"[16]. Metals such as copper, nickel, lead, zinc, and chromium were analyzed using Electro Thermal Atomic Absorption Spectrometry, and specific Measurement conditions were followed using already reported methods [17, 18]. Moreover, iron concentration was detected via Flame Atomic Absorption Spectrometry using specific measurement conditions [19].

RESULTS

Questionnaire responses revealed some consumers reported health issues such as vomiting, acidity, headache, cough, obesity, chest pain, and self-reported diagnoses of cardiovascular or kidney disease. These findings are based on self-reported information and indicate possible associations rather than confirmed clinical causation. 25 oil samples from different canteens of Sindh University were subjected to physicochemical analysis. PH and saponification value were seen to decline in the oil samples after frying. Other parameters like specific gravity, viscosity and acid value increased in the case of after frying oil (AFO) and this increase was found to be statistically significant with a p value<0.050 (Table 1).

Table 1: Determination of Physical and Chemical Properties in BFO and AFO Samples

Sr. No.	Physico-chemical Parameters (n=25)	BFO (Mean ± SD)	AFO (Mean ± SD)	p-value
1	pH	5.15 ± 0.64^{a}, $6.8\text{-}4.71^{b}$	3.70 ± 0.42^{a}, $3.16\text{-}4.46^{b}$	0.230
2	Specific Gravity (g/ml)	0.58 ± 0.05^{a}, $0.51\text{-}0.76^{b}$	0.95 ± 0.01^{a}, $0.91\text{-}0.99^{b}$	0.040*
3	Viscosity (Pa/s)	1.62 ± 0.28^{a}, $1.6\text{-}3.9^{b}$	5.17 ± 1.66^{a}, $1.6\text{-}6.2^{b}$	0.010*
4	Acid Value (mg/KOH/g)	0.54 ± 0.18^{a}, $0.1\text{-}0.6^{b}$	1.40 ± 0.15^{a}, $1.2\text{-}1.9^{b}$	0.020*
5	Saponification Value (mg/KOH/g)	130 ± 7.79^{a}, $115\text{-}138^{b}$	96.4 ± 1.14^{a}, $91\text{-}98^{b}$	0.250

p<0.050* Statically Significant ('a indicates mean values and 'b' indicates minimum and maximum values)

Metal analysis in oil samples depicted that all metals, specifically Nickel, chromium and Lead, were significantly increased (p-value<0.050) in their content after frying oil samples. Whereas only the Zinc content was decreased in oils after frying (p-value>0.050) (Table 2).

Table 2: Determination of Metals in (BFO) and (AFO) Samples

Sr. No	Metals Concentration (mg/L)	BFO (Mean ± SD)	AFO (Mean ± SD)	p-value	Permissible Values of Metals (mg/L)
1	Copper	0.3 ± 0.01^{a} $0.2 \text{-} 0.9^{b}$	2.07 ± 0.3^{a} $0.1\text{-}2.6^{b}$	0.041*	0.1
2	Iron	0.7 ± 0.2^{a} $0.1\text{-}0.4^{b}$	1.24 ± 0.60^{a} $2.1\text{-}2.6^{b}$	0.043*	54
3	Nickel	0.6 ± 0.1^{a} $0.3\text{-}0.9^{b}$	2.43 ± 0.2^{a} $1.2\text{-}2.7^{b}$	0.020*	0.2
4	Lead	1.21 ± 0.30^{a} $1.2\text{-}1.9^{b}$	2.40 ± 0.54^{a} $2.1\text{-}2.6^{b}$	0.040*	0.1 / 2
5	Zinc	0.8 ± 0.45^{a} $0.07\text{–}0.05^{b}$	0.6 ± 0.55^{a} $0.4\text{-}0.8^{b}$	0.350	0.60
6	Chromium	1.30 ± 0.83^{a} $1.1\text{-}1.3^{b}$	2.93 ± 1.55^{a} $2.1\text{-}2.7^{b}$	0.040*	1.30

p<0.005* Statically Significant (' a indicates mean values and 'b' indicates minimum and maximum values).

Metal analysis in blood samples of snack consumers (students) revealed that all the metal concentrations (except Zinc) were increased in consumers as compared to non-consumers (control), and this increase was statistically significant with p-value<0.050 (Table 3).

Table 3: Metal Concentration in Blood Samples

Sr. No.	Metals (mg/L)	Control Mean ± SD (n=30)	Consumers Mean ± SD (n=30)	p-value	Permissible Values of Metals (mg/L)
1	Copper	1.6 ± 0.06^{a}, $1.2\text{-}1.9^{b}$	2.7 ± 0.9^{a}, $2.9\text{-}3.7^{b}$	0.032*	0.59-1.4
2	Iron	690 ± 17^{a}, $590\text{-}680^{b}$	900 ± 105^{a}, $840\text{-}950^{b}$	0.001*	236-614
3	Nickel	1.3 ± 0.08^{a}, $1.2\text{-}1.9^{b}$	2.5 ± 0.8^{a}, $2.1\text{-}2.9^{b}$	0.021*	0.3-0.77
4	Lead	119 ± 12^{a}, $118\text{-}145^{b}$	160 ± 14^{a}, $178\text{-}190^{b}$	0.030*	20-100
5	Zinc	5.8 ± 1.3^{a}, $5.9\text{-}6.9^{b}$	5.4 ± 2.1^{a}, $5.1\text{-}5.8^{b}$	0.071	4.63-7.73

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6	Chromium	1.5 ± 0.03^a, 1.1-1.1^b	2.72 ± 1.1^a, 2.1-2.6^b	0.042*	0.4-1.2

p<0.050* Statically Significant (' a indicates mean values and 'b' indicates minimum and maximum values).

Findings show ash content percentages across different canteens. Pakoras consistently showed higher ash content (%) than samosas across all canteens, suggesting greater mineral residue retention in pakora samples. The highest ash percentage of samosas has been from the English canteen, while pakoras from the central canteen showed more ash content (Figure 1).

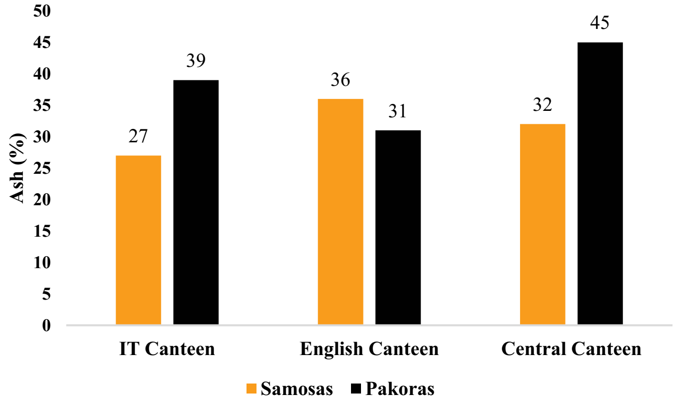

Figure 1: Ash Content (%) of Samosas and Pakoras from Different Canteens

When snacks sold at Sindh University canteens were analyzed for their metal content, it was seen that copper, iron and nickel concentrations were above the permissible limits in samosas whereas lead, zinc and chromium were found in normal ranges in both types of snacks (Table 4).

Table 4: Determination of Metals in Snacks

Sr. No	Metals (mg/kg)	Pakora (Mean ± SD)	Samosa (Mean ± SD)	Permissible Values of Metals (mg/Kg)
1	Copper	18.1 ± 1.31^a, 21-29^b	40.2 ± 13^a, 27-39^b	30
2	Iron	31 ± 2.5^a, 21-24^b	80 ± 19^a, 77-90^b	40-60
3	Nickel	1.39 ± 0.35^a, 1.4-1.9^b	3.69 ± 1.32^a, 2.3-3.7^b	1.5
4	Lead	1.09 ± 0.25^a, 1.2-1.8^b	2.98 ± 0.57^a, 2.4-2.8^b	2.5
5	Zinc	35 ± 1.94^a, 31-39^b	25 ± 1.1^a, 20-29^b	50.0
6	Chromium	19 ± 1.9^a, 14-19^b	4.93 ± 1.55^a 51-59^b	20

p<0.050* Statically Significant. ('a indicates mean values and 'b' indicates minimum and maximum values)

DISCUSSION

Survey results from the questionnaire suggest that reused oil-based snacks can lead to various diseases. Therefore, consumers should opt for fewer fried items in order to save themselves from health complications. Specific gravity, viscosity and acid value tend to increase after more frying (Figure 1). It may be because during the frying process, chemical reactions like thermal reactions, polymerization, oxidation and hydrolysis take place. These reactions give rise to non-volatile, insoluble matter, which increases the viscosity and specific gravity, produces a darker colour and thickens the oil [19]. Our results are in agreement with previous studies, which also reported an increase in these parameters after frying [20, 21]. Acidic values and Saponification values in our investigation after frying oils samples 1.4 and 96.4 were in agreement with those reported by a previous study [22]. Moreover, previous researchers also reported increased concentrations of chromium and nickel after continuous frying of oils in snacks [23]. Our results are similar to previous studies, which also show that metal concentration increased above permissible limits as we subject oil to more frying [23]. The concentration of copper, iron, lead and zinc in our investigated samples of oils was found to be similar to that in previous studies [24]. Particularly, iron toxicity was revealed in consumers' blood after consumption of oily snacks from university canteens. Other metals like chromium, copper, lead and nickel were also not found in permissible limits in consumers' blood. The concentration level of Fe was found to be 690-900 mg/L in the blood of students studying at Sindh University, Jamshoro. Our findings, 600-888 mg/L, are in agreement with the results of previous studies [25]. Furthermore, an increase in copper, iron and nickel concentrations in samosas may be due to add more carbohydrate content due to the use of potato, cooking soda, white flour, salt and red chilli, etc. [26]. This increased metal content is very harmful to our health as it may lead to various disorders like cancer, diabetes, stomach ulcer, kidney disorder, etc. Pakora samples depicted an increase in ash content due to no other ingredient use, only besan in the pakora samples. Other studies have also reported more ash percentage in pakoras [27].

This study was limited by its relatively small sample size and focus on a single university, which may restrict the generalizability of the findings. Future research should expand to multiple institutions and diverse snack types, and consider long-term dietary exposure to toxic metals. Additionally, incorporating advanced analytical techniques could provide more detailed insights into the mechanisms of metal accumulation and aid in developing strategies to mitigate health risks associated with reused frying oils.

CONCLUSION

The present study demonstrates that repeated use of frying oil in snack preparation leads to significant increases in toxic metals (Cu, Fe, Ni, Pb, Cr) in both food products and the blood of consumers, with zinc levels showing a decline. Some detected concentrations exceeded permissible safety limits, indicating a potential risk of chronic metal exposure and associated health effects. Poor hygienic practices during preparation, handling, and storage further compound these risks. Public awareness, strict enforcement of food safety regulations, and discouragement of consuming snacks prepared in repeatedly heated oils are essential to safeguard public health.

Authors' Contribution

Conceptualization: AMS
Methodology: RQ, TGK, FNM
Formal analysis: TGK, BK, FNM
Writing and Drafting: AMS, TGK, BK
Review and Editing: AMS, TGK, BK, FNM

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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