



Biochemical composition of a smokeless tobacco product (NASWAR) used in Pakistan

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ABSTRACT

Objectives: The objective of the study was to investigate the constituents of the most commonly consumed naswar brands from Khyber Pakhtunkhwa, Pakistan.

Methods: A total of 14 most-sold brands of naswar, two from each division in KP were collected and analyzed by gas chromatography-mass spectrometry and inductively coupled plasma mass spectrometry to determine their organic and elemental composition respectively. In addition, pH, ash content, and aflatoxin assessment were also performed.

Results: The average pH and ash content of all the test samples were 8.61 ± 0.271 and 18.94 ± 7.79 % respectively. Overall, the presence and concentration of the different constituents vary across the different naswar brands. GC-MS analysis identified 85 different organic compounds, the commonest being nicotine followed by glycerol tricaprlylate and Di-n-octyl phthalate. Metal ions present in the highest concentration were iron (1146.84 ± 1050.31 mg/kg), tungsten (306.59 ± 494.82 mg/kg), and copper (288.95 ± 77.16 mg/kg). Aflatoxin B1 and B2 were the most common aflatoxins identified in naswar samples.

Conclusions: Overall, naswar contains an array of organic, inorganic, toxic, and heavy metals and aflatoxin constituents with proven toxic and carcinogenic potential. Furthermore, high pH and nicotine contents make it highly addictive. There is an urgent need to enact and implement ST control policies to curb naswar use in Pakistan.

1. Introduction

Smokeless tobacco (ST) use is a growing public health concern with an estimated 360 million users across the globe (The Tobacco Atlas, 2022) of which, more than 90 % live in the South Asian region. Despite being associated with multiple disease conditions (Boffetta and Straif, 2009; Critchley and Unal, 2003), the use of ST products is gradually on the rise in South Asian countries (Chugh et al., 2023). This rise in consumption can be attributed primarily to a lack or poor implementation of ST-specific tobacco control laws and legislation (Azeem et al., 2022). An important contributor to these legislative deficiencies is the poor understanding of the risks associated with the use of these ST products. Compared to smoking tobacco such as cigarettes, there is insufficient scientific evidence on the toxicity of ST products commonly consumed in

developing countries. As a result, these products are considered safe or less harmful (Wackowski et al., 2014) by the general public. The situation is further complicated by the considerable variation in ST products in terms of varieties, their composition, and associated health risks among different countries (Ahmad et al., 2020a). Nonetheless, scientific evidence from across the globe suggests that ST use is a prime risk factor for oral, pharyngeal, and esophageal cancers, coronary heart diseases, and poor birth outcomes and contributes to 0.65 million deaths each year (Siddiqi et al., 2020).

ST use is also common in Pakistan with an estimated 8 % of the population using different ST products, the commonest being Paan, Gutkha, and Naswar (Naz et al., 2018). Naswar use is mostly common among the ethnic Pashtun population of Khyber Pakhtunkhwa (KP) province, wherein around 15 % of the general population uses naswar

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(Khan et al., 2017). In Peshawar, the capital city of KP, around 60 % of the total tobacco users consume naswar (Ahmad et al., 2020a). Naswar is prepared in local cottage industries by mixing sundried tobacco leaves with slaked lime, ash, and flavoring agents (Zakiullah et al., 2012) and packed and sold in small poly-ethylene bags (Sajid and Bano, 2016). Naswar usage for a long time has commonly been linked with oral cancer with pooled estimates suggesting that habitual naswar users are at 10 times higher risk of developing oral cancer than non-users (Suliankatchi et al., 2019). Moreover, a variety of oral (leukoplakia, oral submucous fibrosis, caries, periodontal diseases, teeth staining, and halitosis) (Asthana et al., 2019; John et al., 2021) and systemic (cardiovascular and other) diseases are also reported in habitual naswar users (Khan et al., 2017; Sinha et al., 2016).

Although naswar use is common in KP, scientific studies exploring the composition of products are scarce. To date, the limited available evidence suggests the presence of toxic constituents (nicotine, heavy metals, tobacco-specific nitrosamines) (Ahmad et al., 2020b; Zakiullah et al., 2012) and bacterial contamination in naswar brands in KP (Habib et al., 2023). Storage of naswar may also expose it to fungal contamination (Saleem et al., 2018), and become a potential source of mycotoxins and aflatoxins with known carcinogenic potential in humans (Ahmed et al., 2023). However, efforts to identify aflatoxin presence in naswar have not been carried out as yet.

The scientific studies assessing the composition of naswar were conducted at least a decade ago (Zakiullah et al., 2012) and are not representative of the advances in preparation methods and product composition owing to the greater demand and popularity in the recent past (Ahmad et al., 2020a). Moreover, the composition of naswar products varies among different areas and regions within Pakistan. Additionally, there are brand differences in terms of the amount of tobacco, ash, and slaked lime as well as the type and nature of additives used in the products. It is, therefore, imperative to carry out the compositional analysis of the naswar products available in the market at the moment, to assess the risks associated with their toxicity. The current study aims to comprehensively assess the composition (organic and inorganic constituents), toxic and heavy metals, and microbial toxins (aflatoxins) contamination in the most commonly consumed naswar brands in the KP province of Pakistan.

2. Materials and methods

2.1. Study design and setting

This cross-sectional study was conducted in all 7 divisions (Peshawar, Mardan, Hazara, Kohat, DI Khan, Bannu, and Malakand) of KP. A total of 14 most-sold brands of naswar, two from each division were collected using a purposive sampling technique.

On-site information of all naswar samples was collected using a structured questionnaire. Three freshly prepared samples were purchased, put in zip-locked polypropylene bags, and transported to the lab. The samples were weighed using a calibrated digital balance and the average measure of the three was recorded as the final weight of the pouch contents. The samples were stored at -20°C till further analysis.

2.2. pH determination

The pH was determined on the day of sample collection before storage by adding 2.0 g of the sample into 20 mL of deionized water. All the sample ingredients were thoroughly mixed using a digital shaker and the pH was determined at 5, 15, and 30-minute intervals using a digital pH meter (Orion Star™ A211).

2.3. Ash contents

To determine the ash contents, one full bag of the samples was kept in a pre-dried silica dish and heated in a muffle furnace at $500\text{--}550^{\circ}\text{C}$

till all the organic materials were removed and white color appeared. To ensure complete combustion, each sample was heated for an additional 30 min, and the weight of the ash contents was measured using a digital balance.

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

For GC-MS analysis, naswar samples were extracted in three different solvents (ethanol, chloroform, and N-Hexane). Extraction was performed by adding 50 g of naswar sample in 100 mL of the extraction solvent in a conical flask followed by homogenization for 24hr at room temperature. For three consecutive days, fresh 100 mL of solvent was added into flasks followed by filtration of the extracts using Whatman filter paper ($0.45\ \mu\text{m}$). The final residue was stored at 4°C in the dark. The extracted samples were injected into Shimadzu Gas Chromatograph instrument (GC-2010, Germany) fitted with $30\ \text{mm} \times 0.25\ \text{mm}$ capillary column (Thermo Scientific, U.S.A.), film thickness $0.25\ \mu\text{m}$ and helium gas at a flow rate of $1.2\ \text{mL min}^{-1}$. The initial temperature of the oven was kept at 70°C followed by a 5°C incremental increase in the temperature each minute for 30 min. The instrument chromatogram was coupled with a mass detector Qudrapole (QP 2010 Plus, Shimadzu, Japan) and an Electron Impact ionization (EI) system. The relative percentage amount of each compound was calculated by comparing its average peak area to the total area. The compounds present in the extracts were identified either by the retention time, area, or standards using NIST08 and Wiley 9 built-in libraries.

2.5. Elemental analysis

For elemental analysis, microwave-assisted digestion of naswar samples was carried out using the validated method reported previously (Alhazmi et al., 2018). The digested samples were diluted in deionized water and analyzed via ICP-MS (Agilent, Germany). All samples and standards were analyzed in triplicate using instrument verification standards. The samples were diluted in a 2 % nitric acid and were aspirated using the auto-sampler. Each element concentration was calculated using the dilution values.

2.6. Detection and quantification of aflatoxins

Aflatoxin presence in naswar samples was determined using the AflaTest method (AOAC license no. 940801) as described previously (Swaileh and Abdulkhaliq, 2013). Briefly, 25 g of naswar sample and 5 g of salt (NaCl) were added into a blender jar containing 125 mL of methanol/water (60:40 v/v), blended for one minute, and passed through a filter paper. The resultant filtrate was collected in a clean vessel, diluted with double distilled water, mixed, and filtered again. The filtered extract (5 mL) was passed through an AflaTest and the total Aflatoxins level was determined.

3. Results

3.1. General characteristics of naswar brands

Of all the 14 different naswar brands tested in this study, 6 were in use for more than 20 years and only 5 were tested in the laboratory. The majority of the manufacturers reported grounded tobacco leaves, ash, and slaked lime as the main ingredients to prepare naswar along with some flavoring agents such as menthol and cardamom. The average pH and ash contents of all the test samples were 8.61 ± 0.271 and $18.94 \pm 7.79\%$ respectively (see Table 1).

3.2. Naswar composition

Using GC-MS analysis, 85 different chemical compounds were identified in naswar samples (supplementary Table S1). Representative

Table 1
General characteristics of Naswar samples.

Division	Sample code	Brand in use since	Unit price in PKR	Compositional raw materials	Sources of compositional raw material	Laboratory testing	PH	Ash (%)
Peshawar	S1	2005	20	Local tobacco, ash, slacked lime	Local dealers	No	8.56	10.9
	S2	1997	20	Local tobacco, ash, slacked lime	Local dealers	Yes	8.7	16.4
Mansehra	S3	2008	10	Local tobacco, ash, oil, slacked lime, cardamom	Local dealers	No	8.35	15.1
	S4	2000	10	Local tobacco, ash, slacked lime, flavoring agents	Local dealers	No	8.96	21.1
Dera Ismail Khan	S5	2008	20	Local tobacco, ash, oil, slacked lime, menthol	Local dealers	No	8.12	31.4
	S6	2003	10	Local tobacco leaves, ash, slacked lime, menthol	Local dealers	No	8.59	26.4
Banu	S7	1999	20	Local tobacco leaves, ash, slacked lime, flavoring agents, menthol, cardamom	Local dealer	No	8.39	16.2
	S8	2010	20	Local tobacco leaves, ash, calcium oxide, flavoring agents, menthol, cardamom	Local dealer	No	8.85	20.8
Kohat	S9	1995	20	Local tobacco leaves, wood ash, calcium oxide, flavoring agents, menthol, cardamom	Local dealer	Yes	8.14	10.9
	S10	1997	20	Local tobacco leaves, ash, calcium oxide, flavoring agents, menthol, cardamom	Local dealer	No	8.73	16.0
Mardan	S11	1998	20	Local tobacco leaves, ash, calcium oxide, flavoring agents, menthol, cardamom	Local dealer	Yes	8.7	32.4
	S12	2003	20	Local tobacco leaves, ash, calcium oxide, flavoring agents, menthol,	Local dealer	Yes	8.77	26.9
Swat	S13	NA	10	Local tobacco leaves, ash, calcium oxide, flavoring agents	Local dealer	No	8.93	9.6
	S14	2000	20	Local tobacco leaves, ash, calcium oxide, flavoring agents	Local dealer	Yes	8.8	10.5
							8.61 ± 0.271	18.94 ± 7.79

chromatogram analysis of three naswar samples is presented in [supplementary figure S1](#). Nicotine was the most common constituent present in all samples. Depending on the extraction solvent, the highest area % of nicotine was 97 % in the N-Hexane extract of the S4 naswar sample while the lowest area% (26.1 %) was found in the ethanolic extract of S2. The second highest area% (49.6 %) was observed for the compound glycerol triacrylate which was present only in chloroform extract of sample S2.

Of all the compounds isolated in naswar samples, 23 are classified as hazardous and harmful to humans (Table 2). The majority of the hazardous and toxic constituents were from carboxylic (cotinine, norcotinine, 1,3-Diethylurea) and polyaromatic group of compounds (benzoic acid, 2-ethylhexyl ester, Benzene, 4-ethyl-1,2-dimethyl-). Naswar samples S2 and S3 had the highest number (n = 10) of hazardous compounds followed by S1 and S5 brands (n = 8). Urea, N, N'-diethyl- was the major hazardous compound frequently reported in eight samples ranging from (1.64–9.78 %).

3.3. Elemental composition

Elemental analysis using the ICP-MS method identified twelve elements in all naswar samples (Table 3). Iron was the most common element present in all samples with the highest average concentration ($1146.84 \pm 1050.31 \mu\text{g}/\text{kg}$) followed by tungsten ($306.59 \pm 494.82 \mu\text{g}/\text{kg}$) and copper ($288.95 \pm 77.16 \mu\text{g}/\text{kg}$). Elemental concentration also varied widely among different naswar samples. Lead, one of the most toxic heavy metals was also found in all samples with an average concentration of $190.28 \pm 96 \mu\text{g}/\text{kg}$.

3.4. Aflatoxin contents

Recovery values for the total aflatoxins range from 36 to 84 % for all samples as presented in Table 4. Aflatoxin B1 and B2 were the most common aflatoxins identified in 12 and 11 naswar samples respectively. Aflatoxins G1 and G2 were detected in only 3 and 2 samples. The highest

average concentration of total aflatoxins (8.40 PPB) was observed in S2 samples and S8 and minimum concentration (3.60 PPB) was found in sample S1. Only Aflatoxin B1 was present in S3 and crossed the upper acceptable limit (5 PPB) considered safe for humans. None of the samples crosses the total AFB limit of 10 PPB.

3.5. Carcinogenic compounds identified in naswar samples

Of all the organic and heavy metal elements detected in naswar samples, five constituents are carcinogenic based on the International Agency for Research on Cancer (IARC) classification (IARC, 2023). These include chromium, nickel, tungsten, antimony, and lead. As per IARC classification, nickel and chromium are classified as group 1 compounds that are carcinogenic in humans. Tungsten, antimony, and lead are in group 2A which are probably carcinogenic in humans.

4. Discussion

The current study, thoroughly assessed the biochemical composition of naswar brands commonly used in KP, Pakistan. The overall composition of the samples does not vary considerably across the different regions of the KP province. All the samples possess an alkaline pH ranging from 8.1 to 8.9. These results are in concordance with the findings of a recent research study reporting the basic pH of the ST products commonly consumed in India (Sharma et al., 2023). The higher and alkaline pH of naswar and other ST products is mainly due to the addition of slaked lime and ammonium bicarbonate. The alkaline pH of ST products accelerates the release of unprotonated nicotine and enhances its absorption through the oral mucosa thus increasing the risk of nicotine dependency in users (Pickworth et al., 2014). The average ash contents of the test samples were low (range: 9.6–31.4 %). Since naswar in the KP region is mostly used in moist form, the ash contents are generally low compared to other ST products (Sharma et al., 2023).

On a global scale, the chemical composition of ST products varies widely between different types and also within different brands of the

Table 2
List of hazardous compounds identified in naswar samples.

S#.	List of compounds	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	Hazard classes
1	Tri methyl dodecane													1.9		Skin, and respiratory tract irritation
2	Ethylhexyl benzoate		1.63													Long-term hazard/ Reproductive toxicity
3	Menthol	3.69									0.24			0.69		Skin irritation
4	1-octadecyne	5.09	9.25	0.23	0.32	0.15	0.32							0.76		Warning Specific target organ toxicity/ Respiratory tract irritation
5	Myosmine	1.23	0.25												0.15	Respiratory disorders/ chronic bronchitis
6	Megastigmatrienone	0.47														Colorectal cancer/ Inflammatory bowel/ disease/Eosinophilic esophagitis
7	n.Hexadecanoic acid	1.93	1.79	0.2	0.25	0.52	0.32	0.28	0.22	0.55		0.25		0.76	0.56	Skin/Eye Damage/target organ toxicity/ Respiratory tract irritation
8	2H-tetrazol-5-amine,2-(phenylmethyl)-	8.3										0.22			0.36	Respiratory irritation Eye lungs, thorax damage/irritation/ depression
9	Cotinine	1.3	2.7	1.02		1.95		0.91						2.6	1.05	Sensitization, Skin Narcotic effects/irritatio.
10	Nicotyrine		0.93				0.37			0.21		0.43				target organ toxicity/ Skin corrosion/irritation corrosion/irritation
11	Myristic acid		2.01													Acute toxicity, oral
12	Adipic acid, bis(2-ethylhexyl) ester		10.74													Eye and skin irritation
13	7-tetradecenal,(Z)-														3.23	eye damage/irritation damage fertility
14	Norcotinine		0.18		0.51	0.38		0.35				0.98				Aspiration hazard
15	1,3- diethylurea		2.53													causing cancer
16	Urea, N,N-diethyl-			3.54	9.81	4.66	1.64	5.75	2.81			4.28	9.78			Harmful by inhalation, ingestion or skin absorption
17	Undecane			2.57		1.27	2.1	2.07	1.6	2.27		1.34	1.37			Health hazards
18	Dodecane			1.41		0.78		0.19		1.27		1.28				
19	Benzoic acid, 2-ethylhexyl ester			0.72										5.45		
20	Benzene, 4-ethyl-1,2-dimethyl-			2.69						2.55						
21	Naphthalene			0.83												
22	Tridecane			0.84		0.48				0.79	0.64		0.75	0.55		
23	Anabasine	5.5									0.54					

Table 3
Concentration (µg/kg) of various elements in different Naswar samples as measured by ICP-MS.

Division	Sample	Carbon monoxide	Manganese	Zinc	Tungsten	Rubidium	Copper	Antimony	Iron	Lead	Silver	Nickel	Chromium
Peshawar	S1	15.44	186.48	211.41	168.53	25.58	369.43	24.17	687.64	114.82	214.59	386.18	211.44
	S2	11.49	130.96	118.48	566.90	11.35	277.06	8.18	377.58	63.40	122.66	284.05	117.91
Mansehra	S3	13.92	153.96	330.61	20.40	8.96	339.17	12.36	1196.59	206.70	151.68	341.46	71.51
	S4	8.89	209.65	119.03	52.24	9.89	250.36	81.93	835.22	343.75	287.75	281.63	79.47
Dera Ismail Khan	S5	11.80	181.61	180.35	6.68	7.49	340.95	28.83	920.78	224.14	147.26	320.91	170.77
	S6	23.51	181.00	817.18	1576.09	26.99	454.79	13.41	1242.63	277.95	121.24	283.44	95.53
Banu	S7	12.21	138.24	183.05	1013.77	8.39	269.94	16.31	821.19	193.25	123.61	326.35	67.98
	S8	15.14	353.88	167.54	4.85	23.20	340.75	10.75	4459.93	113.71	70.16	223.61	110.98
Kohat	S9	10.32	173.27	98.97	34.58	8.52	239.88	9.05	1738.26	89.45	103.43	235.09	30.20
	S10	43.42	255.14	10.48	794.93	13.41	196.57	28.94	1643.85	153.90	108.47	90.05	135.88
Mardan	S11	5.05	71.98	30.35	24.98	8.66	178.6	5.30	364.30	224.49	45.56	103.95	18.03
	S12	6.44	126.32	37.85	22.51	7.56	289.03	26.45	886.82	183.53	25.54	151.69	95.08
Swat	S13	6.20	109.66	15.76	0.46	7.95	309.70	176.95	543.85	382.92	33.42	140.21	4.53
	S14	5.27	72.61	23.43	5.40	5.34	188.95	9.67	337.14	91.89	35.79	94.28	8.34
	Mean	13.5 ± 9.9	167.4 ±	167.4	306.5 ±	12.3 ± 7.2	288.9	32.3 ±	1146.8	190.2	113.6	233.0	86.9 ±
	± SD		73.6	±	494.8		± 77.1	45.8	±	± 96.0	± 7.5	±	60.8
					208.3				1050.3			100.3	

same product (Kaur et al., 2019). In the current study, naswar samples also exhibit considerable compositional ingredients that are considered hazardous for human consumption. For example, nicotine, a well-known

alkaloid in tobacco that is linked to addiction and health risks including cardiovascular diseases and cancer (Mishra et al., 2015) was the most common chemical constituent in naswar samples. Similarly, cotinine

Table 4
Aflatoxins concentration (ppb) in naswar samples.

Division	Sample code	Aflatoxins concentration in ppb or µg/kg					Total	Standard	Percent recovery
		B1	B2	G1	G2				
Peshawar	S1	1.40	2.20	ND	ND	3.60	10	36 %	
	S2	2.80	5.60	ND	ND	8.40	10	84 %	
Mansehra	S3	5.60	1.40	ND	ND	7.00	10	70 %	
	S4	1.40	2.80	ND	ND	4.20	10	42 %	
Dera Ismail Khan	S5	1.40	2.80	ND	ND	4.20	10	42 %	
	S6	2.80	1.40	1.40	ND	5.60	10	56 %	
Banu	S7	4.20	ND	ND	ND	4.20	10	42 %	
	S8	ND	ND	2.80	5.60	8.40	10	84 %	
Kohat	S9	1.40	2.80	ND	ND	4.20	10	42 %	
	S10	2.80	2.80	ND	ND	5.60	10	56 %	
Mardan	S11	1.40	5.60	ND	ND	7.00	10	70 %	
	S12	2.80	2.8	ND	ND	5.60	10	56 %	
Swat	S13	ND	ND	1.40	4.20	5.60	10	56 %	
	S14	1.40	2.80	ND	ND	4.20	10	42 %	

and norcotinine, the biomarkers for nicotine exposure in humans (Benowitz et al., 2009) can absorb quickly through the oral or respiratory mucosa, enter the bloodstream, and can even cross the blood–brain barrier (Shoji et al., 2023). Octadecenoic acid (stearic acid), with proven DNA damage and tumorigenic potential (Alhazmi et al., 2019) was also present in some naswar samples. Similarly, polycyclic aromatic hydrocarbons such as naphthalene which is reported in three naswar brands are a respiratory toxicant as well as a possible human carcinogen (Tra-boulsi et al., 2020). We have also found nitrogenous compounds in naswar samples. The accumulation of Tobacco-Specific Nitrosamines (TSNAs) in tobacco products is influenced by factors like nicotine conversion and nitrate content. Nitrates, which are chemically unstable, can react with tobacco alkaloids, either directly or through microbial digestion to form TSNAs (Habib, U. et al., 2023).

ST products that are commonly consumed in Southeast Asian populations contain additional flavoring agents with varying concentration ranges (Lisko et al., 2014). In this study, the identification of menthol in at least three naswar brands confirms the local manufacturer's intentional addition of this flavoring agent into the product. The addition of menthol increases the attraction to new users by making the product more palatable and acts as a reinforcer (Ahijevych and Garrett, 2010). Another interesting finding of this study was the identification of ethylamphetamine carbamate in naswar samples S12. Ethylamphetamine carbamate is an amphetamine derivative and is classified as a drug with addiction and abuse potential. If ingested, it may induce hallucinations, cardiac arrhythmias, blurred vision, and a strong feeling of intoxication (Zanda and Fattore, 2017). The presence of amphetamine in these naswar samples raises important questions. It could indicate adulteration of other plant products containing amphetamines with similar properties. Alternatively, it is plausible that amphetamine, in its chemical form, was directly added to these naswar products given its ready availability in the market (Alhazmi et al., 2019). This important finding warrants further investigations to better understand the sources and health implications of such adulterations.

In addition to the organic constituents, we have also reported twelve different elements, five of which are carcinogenic in humans. These findings are also in concordance with previous reports indicating carcinogenic elements in ST products (Sami et al., 2021). Previously, several studies have identified toxic heavy metals and other hazardous elements in ST products across the world (Brima, 2016; Guezguez et al., 2021; Shetty and Hegde, 2021). It is important to note the metallic composition of individual samples varied widely among the samples reflecting differences in the amount and composition of the ingredients and also the processing, wrapping, and storage of the product. Our study, for the first time, also reported the presence of aflatoxins in naswar products. Previously, AFB1 and AFG1 aflatoxins have been reported in the ST products (Qi et al., 2017). Chronic toxicity of aflatoxins can lead to liver cell carcinoma in humans, stunted growth, weakened

immunity, and cirrhosis in unhealthy individuals (Ahmed et al., 2023). While we were able to identify aflatoxins in naswar samples, the source of contamination whether it is because of direct contamination of tobacco leaves, use of additives, pH, or storage conditions is yet to be known.

Although the current study is the first ever and most comprehensive analysis of the composition of the most famous naswar brands from all the administrative regions of KP, it has some limitations. First, naswar samples used in the study were selected based on popularity, and not all brands were selected thus greatly limiting the generalizability of the study findings. Second, we could not assess the impact of storage and shelf-life variations on naswar composition. Third, the sample size per region may vary considerably but we could only test two samples per region.

5. Conclusion

The present study identified several organic (85), metallic (12) and aflatoxins (4) constituents in naswar samples collected from all administrative regions of KP, Pakistan. Naswar also possess highly addictive potential due to alkaline pH (8.61 ± 0.271) and high nicotine contents. The addictive potential coupled with the presence of carcinogenic compounds renders naswar a very dangerous product for human consumption. Given that naswar is manufactured by the cottage industry in Pakistan, steps must be taken to look into regulating their manufacture and sale.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2024.103168>.

References

- Ahijevych, K., Garrett, B.E., 2010. The role of menthol in cigarettes as a reinforcer of smoking behavior. *Nicotine Tob. Res.* 12, S110–S116. <https://doi.org/10.1093/ntr/ntq203>.
- Ahmad, F., Boeckmann, M., Khan, Z., Zeeb, H., Khan, M.N., Ullah, S., Dreger, S., Haq, Z. U., Forberger, S., 2020a. Implementing smokeless tobacco control policy in Pakistan: a qualitative study among Naswar supply chain actors. *Tob Control tobaccocontrol* 2020-055748. Doi: 10.1136/tobaccocontrol-2020-055748.
- Ahmad, F., Javaid, A., Khan, Z., 2020. SMOKELESS tobacco control in Pakistan. *J. Postgraduate Med. Inst.* 34.
- Ahmed, M.Z., Alqahtani, A.S., Nasr, F.A., Tabish Rehman, Md., Alsufyani, S.A., AlAjmi, M.F., Alhuzani, M.R., 2023. Detection and isolation of aflatoxin producing *Aspergillus* sp. in chewing and smokeless tobacco by microbial and molecular methods. *Saudi J. Biol. Sci.* 103704 <https://doi.org/10.1016/j.sjbs.2023.103704>.
- Alhazmi, H., Ahsan, W., Attafi, I., Khalid, A., Abdelwahab, S., Bratty, M., Sultana, S., 2018. Elemental profiling of smokeless tobacco samples using inductively coupled plasma-mass spectrometry, their chemometric analysis and assessment of health hazards. *Pharmacogn. Mag.* 14, 587–596. <https://doi.org/10.4103/pm.pm.262.18>.
- Alhazmi, H.A., Khalid, A., Sultana, S., Abdelwahab, S.I., Ahsan, W., Oraiby, M.E., Al, B. M., 2019. Determination of phytochemicals of twenty-one varieties of smokeless tobacco using gas chromatography-mass spectrometry (GC-MS). *S. Afr. J. Chem.* 72, 47–54. <https://doi.org/10.17159/0379-4350/2019/v72a7>.
- Asthana, S., Labani, S., Kailash, U., Sinha, D.N., Mehrotra, R., 2019. Association of smokeless tobacco use and oral cancer: a systematic global review and meta-analysis. *Nicotine Tob. Res.* 21, 1162–1171. <https://doi.org/10.1093/ntr/nty074>.
- Azeem, N., Sarfraz, Z., Sarfraz, A., Hange, N., Sarfraz, M., Cherrez-Ojeda, I., 2022. Vaping and smokeless tobacco control in South Asia: a policy review. *Ann. Med. Surg. (Lond.)* 81, 104285. <https://doi.org/10.1016/j.amsu.2022.104285>.
- Benowitz, N.L., Hukkanen, J., Jacob, P., 2009. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb. Exp. Pharmacol.* 29–60 https://doi.org/10.1007/978-3-540-69248-5_2.
- Boffetta, P., Straif, K., 2009. Use of smokeless tobacco and risk of myocardial infarction and stroke: systematic review with meta-analysis. *BMJ* 339, b3060. <https://doi.org/10.1136/bmj.b3060>.
- Brima, E.I., 2016. Determination of metal levels in Shamma (smokeless tobacco) with inductively coupled plasma mass spectrometry (ICP-MS) in Najran, Saudi Arabia. *Asian Pac. J. Cancer Prev.* 17, 4761–4767. <https://doi.org/10.22034/APJCP.2016.17.10.4761>.
- Chugh, A., Arora, M., Jain, N., Vidyasagan, A., Readshaw, A., Sheikh, A., Eckhardt, J., Siddiqi, K., Chopra, M., Mishu, M.P., Kanaan, M., Rahman, M.A., Mehrotra, R., Huque, R., Forberger, S., Dahanayake, S., Khan, Z., Boeckmann, M., Dogar, O., 2023. The global impact of tobacco control policies on smokeless tobacco use: a systematic review. *Lancet Glob. Health* 11, e953–e968. [https://doi.org/10.1016/S2214-109X\(23\)00205-X](https://doi.org/10.1016/S2214-109X(23)00205-X).
- Critchley, J.A., Unal, B., 2003. Health effects associated with smokeless tobacco: a systematic review. *Thorax* 58, 435–443. <https://doi.org/10.1136/thorax.58.5.435>.
- Guezguez, F., Abdelwaheb, M., Anane, I., Rekiq, S., Saguem, S., Charfeddine, B., Rouatbi, S., 2021. Chemical characteristics and cancer risk assessment of smokeless tobacco used in Tunisia (neffa). *Pan Afr. Med. J.* 40 <https://doi.org/10.11604/pamj.2021.40.45.24751>.
- Habib, U., Aziz, T., Sarwar, A., Khan, Z., Shahzad, M., Alharbi, M., Alsahammari, A., 2023. Evaluating the bacterial diversity of smokeless tobacco product using shotgun metagenomic analysis. *Appl. Ecol. Env. Res.* 21, 3045–3056. https://doi.org/10.15666/aeer/2104_30453056.
- IARC, 2023. List of Classifications – IARC Monographs on the Identification of Carcinogenic Hazards to Humans [WWW Document]. URL <https://monographs.iarc.who.int/list-of-classifications/> (accessed 11.21.23).
- John, R.M., Sinha, P., Munish, V.G., Tullu, F.T., 2021. Economic costs of diseases and deaths attributable to tobacco use in India, 2017–2018. *Nicotine Tob. Res.* 23, 294–301. <https://doi.org/10.1093/ntr/ntaa154>.
- Kaur, J., Sharma, A., Kumar, A., Bhartiya, D., Sinha, D.N., Kumari, S., Gupta, R., Mehrotra, R., Singh, H., 2019. SLTChemDB: a database of chemical compounds present in smokeless tobacco products. *Sci. Rep.* 9, 7142. <https://doi.org/10.1038/s41598-019-43559-y>.
- Khan, Z., Dreger, S., Shah, S.M.H., Pohlabeln, H., Khan, S., Ullah, Z., Rehman, B., Zeeb, H., 2017. Oral cancer via the bargain bin: the risk of oral cancer associated with a smokeless tobacco product (Naswar). *PLoS One* 12, e0180445.
- Lisko, J.G., Stanfill, S.B., Watson, C.H., 2014. Quantitation of ten flavor compounds in unburned tobacco products. *Anal. Methods* 6, 4698–4704. <https://doi.org/10.1039/C4AY00271G>.
- Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., Garg, A., 2015. Harmful effects of nicotine. *Indian J. Med. Paediatr. Oncol.* 36, 24–31. <https://doi.org/10.4103/0971-5851.151771>.
- Naz, S., Naz, S., Nadeem Saqib, M.A., Bashir, F., Rafique, I., 2018. Prevalence of smokeless tobacco use in Pakistan: insight from the global adult tobacco survey Pakistan (GATS Pakistan-2014). *J. Pak. Med. Assoc.* 68 (Suppl. 2), S7–S12.
- Pickworth, W.B., Rosenberry, Z.R., Gold, W., Koszowski, B., 2014. Nicotine absorption from smokeless tobacco modified to adjust pH. *J. Addict. Res. Ther.* 5, 1000184. <https://doi.org/10.4172/2155-6105.1000184>.
- Qi, D., Fei, T., Liu, H., Yao, H., Wu, D., Liu, B., 2017. Development of multiple heart-cutting two-dimensional liquid chromatography coupled to quadrupole-orbitrap high resolution mass spectrometry for simultaneous determination of aflatoxin B1, B2, G1, G2, and ochratoxin A in snus, a smokeless tobacco product. *J. Agric. Food Chem.* 65, 9923–9929. <https://doi.org/10.1021/acs.jafc.7b04329>.
- Sajid, F., Bano, S., 2016. Pro inflammatory interleukins and thyroid function in Naswar (dipping tobacco) users: a case control study. *BMC Endocr. Disord.* 16, 1–6. <https://doi.org/10.1186/s12902-016-0127-5>.
- Saleem, S., Naz, S.A., Shafique, M., Jabeen, N., Ahsan, S.W., 2018. Fungal contamination in smokeless tobacco products traditionally consumed in Pakistan. *J. Pak. Med. Assoc.* 68, 1471–1477.
- Sami, A., Elimairi, I., Patangia, D., Watkins, C., Ryan, C.A., Ross, R.P., Stanton, C., 2021. The ultra-structural, metabolomic and metagenomic characterisation of the sudanese smokeless tobacco ‘Toombak’. *Toxicol. Rep.* 8, 1498–1512. <https://doi.org/10.1016/j.toxrep.2021.07.008>.
- Sharma, P., Cheah, N.P., Kaur, J., Sathiy Kumar, S., Rao, V., Morsed, F.A., Choo, M.Y.B., Murthy, P., 2023. Physical and chemical characterization of smokeless tobacco products in India. *Sci. Rep.* 13, 8901. <https://doi.org/10.1038/s41598-023-35455-3>.
- Shetty, S., Hegde, V., 2021. Estimation of toxic metals in smokeless tobacco products. *Tob. Induc. Dis.* 19 <https://doi.org/10.18332/tid/140969>.
- Shoji, T., Hashimoto, T., Saito, K., 2023. Genetic regulation and manipulation of nicotine biosynthesis in tobacco: strategies to eliminate addictive alkaloids. *J. Exp. Botany* erad341. <https://doi.org/10.1093/jxb/erad341>.
- Siddiqi, K., Husain, S., Vidyasagan, A., Readshaw, A., Mishu, M.P., Sheikh, A., 2020. Global burden of disease due to smokeless tobacco consumption in adults: an updated analysis of data from 127 countries. *BMC Med.* 18, 222. <https://doi.org/10.1186/s12916-020-01677-9>.
- Sinha, D.N., Abdulkader, R.S., Gupta, P.C., 2016. Smokeless tobacco-associated cancers: a systematic review and meta-analysis of Indian studies. *Int. J. Cancer* 138, 1368–1379. <https://doi.org/10.1002/ijc.29884>.
- Suliankatchi, R.A., Sinha, D.N., Rath, R., Aryal, K.K., Zaman, M.M., Gupta, P.C., Karki, K. B., Venugopal, D., 2019. Smokeless tobacco use is “replacing” the smoking epidemic in the South-East Asia region. *Nicotine Tob. Res.* 21, 95–100. <https://doi.org/10.1093/ntr/ntx272>.
- Swaleih, K.M., Abdulkhalig, A., 2013. Analysis of aflatoxins, caffeine, nicotine and heavy metals in Palestinian multifloral honey from different geographic regions. *J. Sci. Food Agric.* 93, 2116–2120. <https://doi.org/10.1002/jsfa.6014>.
- The Tobacco Atlas, 2022. The Global Impact of Smokeless Tobacco [WWW Document]. Tobacco Atlas. URL <https://tobaccoatlas.org/challenges/smokeless/> (accessed 2.7.24).
- Traboulsi, H., Cherian, M., Abou Rjeili, M., Preteroti, M., Bourbeau, J., Smith, B.M., Eidelman, D.H., Baglole, C.J., 2020. Inhalation toxicology of vaping products and implications for pulmonary health. *Int. J. Mol. Sci.* 21, 3495. <https://doi.org/10.3390/ijms21103495>.
- Wackowski, O.A., Lewis, M.J., Delnevo, C.D., Ling, P.M., 2014. Smokeless tobacco risk comparison and other debate messages in the news. *Health Behav. Policy Rev.* 1, 183–190. <https://doi.org/10.14485/HBPR.1.3.2>.
- Zakiullah, Saeed, M., Muhammad, N., Khan, S.A., Gul, F., Khuda, F., Humayun, M., Khan, H., 2012. Assessment of potential toxicity of a smokeless tobacco product (naswar) available on the Pakistani market. *Tob. Control* 21, 396–401. <https://doi.org/10.1136/tc.2010.042630>.
- Zanda, M.T., Fattore, L., 2017. Chapter 29 - Novel psychoactive substances: a new behavioral and mental health threat. In: Watson, R.R., Zibadi, S. (Eds.), *Addictive Substances and Neurological Diseases*. Academic Press, pp. 341–353. <https://doi.org/10.1016/B978-0-12-805373-7.00029-3>.