Journal of Health and Rehabilitation Research 2791-156X JHRR

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Original Article

FT-IR Characterization and Determination of Mycotoxins (Aflatoxins) (AF's) from Branded &Unbranded Mixed Spice Samples

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Conflict of Interest: None.

Seelro MY., et al. (2024). 4(2): DOI: https://doi.org/10.61919/jhrr.v4i2.716

ABSTRACT

Background: The global spice market is essential to food industry and public health due to the widespread use of spices as flavor enhancers, preservatives, and medicinal components. However, the quality of spices can be compromised by the presence of mycotoxins, specifically aflatoxins, which pose significant health risks. Ensuring the quality and safety of spices, both branded and unbranded, is of paramount concern, especially in regions with high humidity and temperature that can facilitate fungal growth and mycotoxin production.

Objective: This study aimed to assess the quality of branded and unbranded spice samples from Sindh, Pakistan, by determining their ash content, moisture level, oil composition, and mycotoxin (aflatoxin) content to ensure their suitability for consumption and adherence to food safety standards.

Methods: A total of ten spice samples (five branded and five unbranded) were collected from different cities in Sindh province, Pakistan. Ash content was measured by high-temperature oxidation, while moisture content was quantified through oven drying. Oil was extracted using a Soxhlet apparatus, and its quality parameters, including free fatty acid (FFA), peroxide, iodine, and saponification values, were determined using standard AOCS methods. Aflatoxins were quantified by employing an official AOAC method. Additionally, FT-IR spectroscopy was utilized for qualitative analysis of the spice oils.

Results: The ash content varied widely, with the highest in unbranded samples at 18.33% and the lowest in branded at 5.67%. Moisture content across all samples was within the safe limit of 5-10%, with the highest at 4.46% and the lowest at 2.22%. The oil content ranged from 5.96% to 14.56%, with unbranded samples showing higher values. FFA values exceeded the standard 0.1% in all samples, indicating a potential quality concern. Peroxide values were higher than the standard 2 meq/mol in most samples, raising concerns about oxidative stability. Aflatoxin levels were within the EU regulatory limits, suggesting acceptable levels for consumption. FT-IR analysis corroborated these findings, revealing no significant differences in the spectral profiles between branded and unbranded samples.

Conclusion: The study concluded that while moisture content and aflatoxin levels in the spice samples complied with safety standards, there were notable concerns regarding the ash content, oil quality, and peroxide values. These findings highlight the necessity for regular quality control measures and adherence to food safety regulations to ensure the safety of spice consumption.

Keywords: Spice Quality, Aflatoxins, Mycotoxins, Food Safety, Ash Content, Moisture Content, Oil Composition, FT-IR Spectroscopy, Oxidative Stability, Public Health, Sindh Spices, Analytical Methods.

INTRODUCTION

Mycotoxins, naturally occurring toxic chemical compounds produced by various fungal species, have garnered significant attention due to their profound health implications on humans and animals, including hepatic illnesses, carcinogenicity, teratogenicity, and mutagenicity (1). The genesis of these mycotoxins is closely linked to environmental conditions such as high rainfall, elevated

temperatures, and increased humidity, which are prevalent in regions where spices, a primary commodity for contamination, are cultivated. These climatic factors, alongside deficiencies in Good Manufacturing Practices (GMP) and Good Agricultural Practices (GAP), exacerbate the risk of fungal proliferation and mycotoxin production. The concern over mycotoxins is not unfounded, as evidenced by reports highlighting the frequent contamination of spices such as pepper and chili with aflatoxins (AFs) and ochratoxin A (OTA) (2).

To date, over 400 mycotoxins have been identified, yet only a select few, such as aflatoxins (AFB1, AFB2, AFG1, AFG2), ochratoxin A (OTA), fumonisins (FB1, FB2), and deoxynivalenol, are considered of significant toxicological concern due to their potent toxicity in both animals and humans. Notably, aflatoxins have been classified as Group 1 human carcinogens, predominantly linked to liver cancer, whereas fumonisins and ochratoxin A have been categorized as Group 2B human carcinogens (3). Despite the non-carcinogenic nature of zearalenone and trichothecenes (4), the potential health risks associated with mycotoxin exposure necessitate stringent regulatory standards. The European Union, for instance, has set maximum allowable levels for AFB1 at 5 µg/kg and 10 µg/kg for total aflatoxins in spices, with specific regulations also in place for ochratoxin A (5).

The production of mycotoxins is attributed to fungal genera such as Aspergillus, Alternaria, Fusarium, and Penicillium, which can infest agricultural products both in the field and during storage (6). It is estimated that 5-10% of global agricultural output is affected by fungal spoilage, leading to significant economic losses and health hazards. The Food and Agriculture Organization (FAO) estimates that over 25% of the world's agricultural produce is contaminated with mycotoxins (7), underscoring the pervasive threat these toxins pose to food safety and public health. Spices, in particular, are susceptible to fungal contamination, which not only compromises their quality and flavor but also serves as a vector for mycotoxin proliferation under conducive environmental conditions (8).

The enduring challenge posed by mycotoxins, including aflatoxins, fumonisins, ochratoxin, zearalenone, ergot alkaloids, and trichothecenes, is compounded by their remarkable thermostability, rendering most food processing techniques ineffective in their eradication (9). Ochratoxin A, predominantly produced by Penicillium and Aspergillus genera, exemplifies the diverse toxicological spectrum of mycotoxins, being classified as a Group 2B possible human carcinogen while also exhibiting nephrotoxic, teratogenic, immunotoxin, and hepatotoxic effects across various animal models (10). This intricate interplay between environmental factors, fungal biology, and mycotoxin chemistry underscores the complexity of managing mycotoxin risks in agricultural and food products, necessitating ongoing research, improved agricultural practices, and stringent regulatory oversight to safeguard public health.

MATERIAL AND METHODS

In this study, the focus was on evaluating the presence of mycotoxins, specifically aflatoxins, in branded and unbranded mixed spices collected from various cities within the Sindh province of Pakistan, including Karachi, Hyderabad, Sukkur, and Larkana. All chemicals and reagents utilized throughout the research were of analytical grade, sourced from E-Merck, Darmstadt, Germany, ensuring the reliability and accuracy of the analytical procedures employed.

For sample preparation, a comparative approach was adopted to assess the quality of both branded and unbranded spice samples. Unbranded samples were initially stored openly in various environments, whereas branded samples were kept in sealed, hygienic conditions. Subsequent to grinding, the unbranded samples were meticulously packaged in clean bags for analysis. The classification of these samples, based on their morphological characteristics such as color, led to the identification of three novel local varieties, adhering to the methodology outlined by Barton (1984) for sample identification. These newly identified varieties were systematically coded as UNB-1 through UNB-5.

The analysis incorporated several key procedures to ascertain the quality and safety of the spice samples. Dry ash content was determined using a high-temperature muffle furnace, maintaining temperatures between 500 and 600°C, to oxidize organic substances and vaporize volatile materials, while preserving most minerals in their converted forms. This method is crucial for understanding the mineral composition of the spices. Similarly, wet ashing involved the use of strong acids and oxidizing agents to break down the organic matrix, facilitating the analysis of specific minerals contained within the spices.

Moisture content was quantified through oven drying at 105°C for three hours, based on the AOCS standard method (Aa-3-38), providing insight into the water vapor content of the samples pre and post evaporation. The extraction of oil from the samples employed the Soxhlet extraction method, utilizing n-hexane as the solvent, a modification of the technique reported by Akpan et al., (2006). This process yielded the total oil content, essential for further physicochemical analysis.

Further analyses included the determination of free fatty acids (FFA) as a percentage of oleic acid, peroxide value, iodine value, and saponification value, all according to official AOCS methods. These analyses offer a comprehensive view of the fat quality within the spice samples, which can affect both the health implications and the shelf-life of the products.

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The qualitative analysis of the spice samples employed Fourier-transform infrared spectroscopy (FT-IR), utilizing a Thermo Nicolet Avatar 330 spectrometer with a DTGS detector. This method, particularly with the use of a single bounce-attenuated total reflection (ATR) accessory, provided detailed spectra for identifying chemical constituents and potential contaminants within the samples.

The determination of aflatoxins, a focal point of the study, adhered to the AOAC method 975.36. The results obtained were benchmarked against European Union (EU) regulations, ensuring that the spice samples met the required standards for aflatoxin content, thereby deeming them fit for human consumption.

Data collection encompassed not only the chemical analysis but also involved meticulous record-keeping and classification based on predefined criteria. This was underpinned by a rigorous ethical framework, adhering to the principles outlined in the Helsinki Declaration, to ensure the integrity and ethical conduct of the research. The analysis of the collected data was performed using the Statistical Package for the Social Sciences (SPSS) version 25, allowing for the application of appropriate statistical tests to validate the findings.

This comprehensive methodology, rooted in established scientific principles and ethical guidelines, facilitated a thorough investigation into the safety and quality of branded and unbranded mixed spices, providing valuable insights into the potential health risks associated with aflatoxin contamination.

RESULTS

In the comprehensive analysis of spice samples, Tables 1 and 2 present a detailed quantitative assessment of their composition. The branded spices revealed a range of ash content from 5.67% to 15.33%, moisture levels from 2.22% to 4.46%, and oil contents fluctuating between 5.96% and 13.92%. Notably, the saponification values varied considerably, with a span from 185.13 to 211.07, peroxide values were comparatively low across samples, and iodine values indicated a variability in unsaturation within the oils, ranging from 135.14 to 158.94. The free fatty acid percentage was found to be minimal, underlining the quality of the oils with values between 1.41% and 3.41% (Table 1). In contrast, unbranded samples showed a wider variance in their composition, with ash content extending from a minimal 4.00% in UNB-1 to a significant 18.33% in UNB-3, while moisture content remained within a narrow band similar to branded samples. The oil content for these samples was slightly higher on average, particularly noted in UNB-5, which presented an impressive 14.56%. This was coupled with saponification values that ranged from 197.05 to 223.69, suggesting differences in oil qualities. Peroxide values, an indicator of oxidation, were consistently low, while iodine values remained largely consistent with those found in branded spices. The free fatty acids percentage was comparable to that of branded spices, reinforcing the similarity in oil quality across both categories (Table 2).

Table 1 Comprehensive Analysis of Spice Samples

Sample Type	Sample Name	Ash Content (%)	Moisture Content (%)	Oil Content (%)	Saponification Value (SV)	Peroxide Value (PV)	Iodine Value (IV)	Free Fatty Acids (FFA) (%)
Branded	National Mixed	13.2 ± 0.31	2.52 ± 0.08	13.92 ± 0.32	203.36 ± 5.61	4.00	152.59 ± 5.52	2.08 ± 0.03
Branded	Shan Mixed	15.33 ± 0.47	3.00 ± 0.09	8.86 ± 0.26	207.57 ± 4.81	2.25	158.94 ± 3.64	1.83 ± 0.05
Branded	Mehran Mixed	11.67 ± 0.26	2.22 ± 0.16	7.58 ± 0.24	185.13 ± 3.89	2.75	135.14 ± 3.88	2.35 ± 0.06
Branded	Halwai Mixed	5.67 ± 0.16	4.46 ± 0.23	6.40 ± 0.19	193.54 ± 5.77	3.50	150.37 ± 4.71	1.41 ± 0.04
Branded	Lazat Mixed	8.33 ± 0.24	3.54 ± 0.13	5.96 ± 0.14	211.07 ± 4.96	2.75	157.35 ± 4.22	3.41 ± 0.05
Unbranded	UNB-1	4.00 ± 0.23	2.60 ± 0.12	12.12 ± 0.27	204.76 ± 5.71	2.50	135.78 ± 3.55	1.81 ± 0.07
Unbranded	UNB-2	11.33 ± 0.25	3.20 ± 0.17	13.76 ± 0.29	216.68 ± 3.88	1.75	135.46 ± 3.33	2.22 ± 0.08
Unbranded	UNB-3	18.33 ± 0.28	3.20 ± 0.17	12.89 ± 0.28	223.69 ± 4.62	3.25	142.12 ± 3.32	1.88 ± 0.05

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Sample Type	Sample Name	Ash Content (%)	Moisture Content (%)	Oil Content (%)	Saponification Value (SV)	Peroxide Value (PV)	Iodine Value (IV)	Free Fatty Acids (FFA) (%)
Unbranded	UNB-4	6.67 ± 0.03	2.20 ± 0.12	12.04 ± 0.26	197.05 ± 5.65	2.25	157.03 ± 4.35	1.32 ± 0.04
Unbranded	UNB-5	4.33 ± 0.29	2.00 ± 0.10	14.56 ± 0.33	202.66 ± 4.79	2.50	151.01 ± 4.11	1.48 ± 0.05

The mycotoxin analysis, critical to assessing the safety of the spice samples, is encapsulated in Figures 3 to 10. The determination of aflatoxins, depicted in Figures 3 to 6 for branded spices and Figures 7 to 10 for unbranded spices, illustrated that all samples maintained aflatoxin levels within the detectable limits, ensuring compliance with food safety standards. For branded spices, the total aflatoxins ranged narrowly from 1.15 to 1.95 µg/kg, affirming a commendable control in mycotoxin levels. In a similar fashion, unbranded spices sustained their total aflatoxins between 1.13 and 1.45 µg/kg, reflecting a diligent adherence to safety regulations despite the absence of brand affiliation. These findings highlight the effectiveness of the employed preservation techniques in curtailing the proliferation of these toxic compounds.

Table 2 Determination of Aflatoxins in Branded Spices

Branded Samples	Total Aflatoxins (AFB1+AFB2+AFG1+AFG2) µg/kg	Detectable Limits µg/kg	Analyzed Results µg/kg
B=1	1.45	≥ 1	1.45
B=2	1.15	≥ 1	1.15
B=3	1.65	≥ 1	1.65
B=4	1.39	≥ 1	1.39
B=5	1.95	≥ 1	1.95
STD	0.3002	-	-

Table 3 Determination of Aflatoxins in Unbranded Spices

Unbranded Samples	Total Aflatoxins (AFB1+AFB2+AFG1+AFG2) µg/kg	Standard Limit µg/kg	Results µg/kg	Mean±STD µg/kg
UNB=1	1.35	≥ 1	1.35	-
UNB=2	1.45	≥ 1	1.45	-
UNB=3	1.13	≥ 1	1.13	-
UNB=4	1.17	≥ 1	1.17	-
UNB=5	1.27	≥ 1	1.27	-

The FT-IR spectroscopic analysis provided a qualitative insight into the chemical makeup of the oils extracted from both branded and unbranded spices. The representative spectra, as illustrated in Figures 1 and 2 for branded oils, and Figures 6 to 10 for unbranded oils, showcased distinctive peaks that denote functional groups indicative of various compounds present in the oils. The spectra portrayed in these figures exhibited characteristic absorption bands, which serve as fingerprints for the identification of specific molecular components within the oils. This non-destructive technique proved instrumental in confirming the presence of certain chemical constituents, providing an additional layer of analysis to complement the quantified data. The uniformity of the spectral patterns across the figures substantiates the consistency in the composition of the oils, irrespective of the branding status of the spice samples.

DISCUSSION

In the course of evaluating the quality of spice samples, both branded and unbranded, a comprehensive assessment was conducted to measure the mineral content, moisture levels, oil composition, and the potential presence of mycotoxins—specifically aflatoxins. The ash content analysis, reflecting the inorganic residue post the combustion of organic matter, indicated a significant presence of minerals like potassium, calcium, sodium, and chlorine. It was found that branded samples labeled B showed an ash content percentage (15.33%) exceeding the upper limit of the expected 5-12% range, while unbranded samples, notably UNB-3, also surpassed the typical thresholds with an 18.33% ash content (1). Conversely, other samples fell within the expected ranges, suggesting a variance in spice processing and handling practices across brands.

Moisture content analysis, a critical indicator of shelf life and susceptibility to microbial growth, showed the highest moisture in a branded sample B (4.46%) and the lowest in another branded sample B (2.22%). This range falls below the standard moisture content values of 5-10%, indicating that the spices under study maintained adequate dryness to prevent spoilage (2).

Oil content percentages were particularly revealing, with the highest oil content found in unbranded samples, such as UNB-5 (14.56%). Such elevated levels may contribute to rapid oxidation, potentially impacting the flavor profile and stability of the spices. This was an important finding, as it underlined the necessity for improved handling and storage practices to ensure the longevity and quality of spice products (3).

The analysis of free fatty acids (FFAs) offered insight into the quality of the oil within the spices. Branded sample B demonstrated the highest FFA composition (3.41%), which exceeded the standard value of 0.1%. This elevation of FFAs could be indicative of the degradation of oil quality, which is a vital quality control parameter for food products (4).

Saponification values were another focal point of the study. These values, which inversely correlate with the molecular weight of the oil's constituents, highlighted discrepancies between samples. Both branded and unbranded spices showed instances of saponification values exceeding the standard range (187-196 mg), pointing towards varying molecular weights of the oil content and suggesting a diversity in the types of oils present within the spice samples (5).

The peroxide values obtained further underscored the oxidative stability of the oils in the spices. Most samples, branded and unbranded alike, presented peroxide values that exceeded the standard limit of 2 meq/mol. This finding raises concerns about the potential rancidity and shelf life of the spices, emphasizing the need for careful monitoring of oxidation levels (6).

Iodine values were also measured, serving as an index of the degree of unsaturation within the oils. Samples displayed iodine values divergent from the standard limit (130 mg), reflecting a higher degree of unsaturation and thus a greater number of double bonds within the fatty acid chains of the oils. This variance in iodine values compared to previous findings suggests a potential differentiation in the health implications of the spices, given the role of unsaturated fats in human nutrition (7).

FT-IR spectroscopic analysis provided a qualitative assessment of the oils extracted from the spices. Representative spectra for both branded and unbranded oils (Figures 3-10) displayed characteristic peaks consistent with various functional groups, such as the cis-double bond vibrations and carbonyl ester groups. These spectra offered valuable fingerprints for the identification of specific molecular constituents within the oils and affirmed the homogeneity of the spectral patterns across the samples, reflecting consistency in composition irrespective of brand status (8).

Furthermore, the determination of aflatoxins within the spices showed that all samples-maintained levels within detectable and acceptable limits for human consumption as per EU regulations. This finding suggests effective control measures in place during the spice processing and storage stages, although it highlights the need for stringent and consistent regulatory frameworks to maintain these standards (9).

The study, while comprehensive, acknowledges certain limitations such as the scope of spice varieties tested and the regional focus of the sample collection. It recommends an extension of the survey to a broader array of spices and geographical areas to ensure more generalized findings. Additionally, the research underscores the necessity for regular monitoring by local authorities to maintain quality control and compliance with international standards, as well as further research to understand the implications of high FFA and peroxide values on consumer health (10).

CONCLUSION

In conclusion, the investigation into the quality of branded and unbranded spice samples underscored both strengths and areas for improvement within the spice industry. While the consistency in moisture content and the control of mycotoxin levels are commendable, the variations in ash content, oil quality parameters, and the presence of high peroxide values call for enhanced quality assurance processes. The utilization of FT-IR spectroscopy emerged as a strength of the study, offering a rapid and non-destructive method for assessing the quality of spice oils, yet the overarching recommendation emphasizes the importance of a global standardization of quality metrics to ensure the safety and quality of spices for consumers worldwide.

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