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## Characterization of Microbial and Chemical Properties of Wheat Flour Samples in Hamadan, Iran: A Screening and Investigation Study

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### ABSTRACT

**Background:** The production and use of any food must be done according to existing national and international standards. This study aims to investigate the chemical and microbiological properties of flour samples in Hamadan province of Iran. **Methods:** The results of microbial and chemical tests of 432 flour samples were collected in one of the partner Institute of Standards and Industrial Research of Iran in Hamadan city, and after sorting the data, it was entered into the relevant statistical software and analyzed with appropriate statistical tests. The results were compared with the existing national standards to determine their quality. The evaluated parameters include chemical parameters such as pH, protein, gluten, moisture, and ash) and microbial (mold and total viable count (TVC)). All of the parameters were assessed by the AOAC method. **Results:** The samples had a higher total count of microorganisms than the standard limit set by the Iran National Standards Organization (INSO). The mean value of chemical parameters (pH, protein, gluten, moisture, and ash) were in the national standard ranges. The results of the present study showed that the level of contamination of the samples was according to the national standards, which could pose no risk to consumers' health. **Conclusions:** The results of the microbial and chemical tests were within the Institute of Standards and Industrial Research of Iran. The results of the present study can be used by health authorities to evaluate flour quality across Iran and design further investigations on food products.

### Introduction

Wheat is the most important agricultural product in the human diet food chain (Karizaki, 2017). According to the Foreign Agricultural Service (FAS), global wheat

production in 2022-2023 reached 789.56 million tons (U.S.department of agriculture, 2024). Wheat provides 70% of the world's cereals and is the most widely consumed cereal, and due to pre- and post-

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harvest operations, there are many changes in its physicochemical properties (Kumar *et al.*, 2020).

Additionally, wheat is the primary cereal crop in the human diet and is vital to nutrition globally (Pirhadi *et al.*, 2020). It contains many lipids, vitamins, dietary fiber, protein, antioxidants, micronutrients, etc., which affect its properties (Karizaki, 2017). Scientific research data shows that whole grains effectively prevent cancer, obesity, and some chronic diseases such as coronary heart disease and diabetes (Kumral, 2015). The chemical composition of cereal grains affects the performance of technological features. Also, crushing, sieving, and grinding process creates several types of flour (Cardoso *et al.*, 2019, Pirhadi *et al.*, 2020). The presence of high prolamine and glutenin, which are ingredients of gluten in dough products significantly affects the rheological properties of the dough, the production process, and the final quality of gluten-free products. Gluten-free dough has fewer elastic properties than wheat flour dough, is very loose and difficult to work with, and is similar to cake dough. Due to the low storage power of carbon dioxide, the volume of its products is small (Cardoso *et al.*, 2019).

Generally, wheat flour mainly comprises macronutrients such as starch, water, and proteins, and other micronutrients such as non-starch polysaccharides, lipids, and ash. The type of flour, categorized by its ash content, primarily ensures the production of white or darker crumb bread. While microorganisms do not thrive in such low water activity levels (0.491–0.619), foodborne bacteria and fungi can easily contaminate flour and remain viable for extended periods. Additionally, low moisture levels are known to enhance the heat resistance of foodborne pathogens. Research from Australia, Europe, and North America has identified the presence of *Salmonella* spp., *Escherichia coli*, *Bacillus cereus*, and other spoilage microorganisms in flour. Furthermore, outbreaks of salmonellosis have been linked to the consumption of low-moisture foods, including wheat flour (Condon-Abanto *et al.*, 2016).

This study aims to investigate the microbial and

chemical properties of wheat flour samples including Barbari, Lavash, Taftoon, and Sangak flour taken from Hamadan province in 2019-2022, Iran.

## Materials and Methods

Chemical and microbiological analysis was carried out on 432 samples of types of wheat flour from 2019 to 2022. All microbial (mold and total viable count) and chemical (pH as well as moisture, protein, gluten, and ash contents) properties were assessed by the Official Methods of Analysis (AOAC) that will be explained. Samples were collected and tested according to the Iranian Institute of Standards and Industrial Research guidelines, and SPSS 24 software was used to analyze data.

### Chemical evaluation

All physicochemical parameters in the bread flours were analyzed according to the Iranian National Standards Organization (INSO) protocol, and the details are provided.

### Ash content

Wheat flour ash content was measured via dry ash method. The flour sample was measured into ash dishes; then, the samples were placed in a furnace at 500-600 °C. Flour samples were incinerated until they turned into light gray ash. The samples were weighed after the cooling process, and the ash content was calculated (Marshall, 2010).

### Moisture content

The air-oven method was used to analyze flour moisture. The technique included heating a small fraction of the flour sample for 1 hour at 130 °C. Then, the flour sample was weighed, and the loss in weight before and after heating was considered as moisture content (Nielsen, 2010).

### Protein and gluten and sedimentation

The Zeleny sedimentation test evaluated flour's gluten and protein content. Flour samples were prepared by Zeleny mill, and the measurement was carried out by recording the amount of sedimentation. Obtained numbers via dividing the sedimentation value by the percentage of proteins

is called Zeleny-specific sedimentation value, which describes the baking quality (Tömösközi *et al.*, 2009).

#### pH determination

10 g of samples were mixed with 100 ml of distilled water. After settling the solution context, the pH was measured using an electric pH.

#### Microbiological analysis

**Bacterial analysis:** Total viable count (TVC) was counted using plate count AGAR (PCA). Flour sample dilution in ten-fold with sterile 0.1% media was prepared and spread on the surface of PCA. The plates were incubated (24 h at 37 °C), and the forming unit (cfu/g) was enumerated (Ibeanu *et al.*, 2015).

**Mold analysis:** Samples from wheat flour were cultured on the surface of DG18 (Dichloran Glycerol 18% Agar) mediums. After incubation for 48 h at room temperature, the plates were assessed for mold (fungi and yeast) growth. The quantity of mold was calculated based on Enumerated colony-forming units (CFU/g) (Rose *et al.*, 2012).

#### Data analysis

Samples were collected and tested according to the Iranian Institute of Standards and Industrial

Research guidelines. SPSS 24 software was used to analyze this data. The significance level was considered below 0.05. Mean and standard deviations were used to describe continuous variables, and frequency and percentage were used for categorical variables. A t-test was used to compare chemical and microbial factors with the standard limit (Arifin, 2017).

#### Results

Chemical and microbial analysis were conducted on wheat flour samples collected from 2019 and 2022. The results were compared to the regulatory standards established by the Iranian National Standards Organization (INSO).

**Tables 1-3** demonstrate INSO's acceptable ranges for chemical parameters in different flour types, the chemical characteristics of flour samples and the flour samples microbial counts, respectively. **Figures 1** and **2** represent the chemical and microbial data, respectively. **Figure 3** compares key chemical parameters (pH, protein, moisture, gluten, and ash) to INSO reference values (pH: 5.6-6.5; ash: 0.851-1.225%; protein: ≥11%; gluten: ≥25%; moisture: ≤14.2% [95% CI]; TVC: ≤10<sup>5</sup> CFU/g; mold: ≤5 × 10<sup>3</sup> CFU/g).

**Table 1.** The national standard limit of different types of flour (%).

Types of flour	Protein	Gluten	Moisture	Ash	pH
Barbari flour	11	26	14.2	0.701- 0.850	2.4
Lavash flour	11	25	14.2	0.851-1.225	3.5
Taftoon Flour	11	25	14.2	0.851-1.225	3.5
Sangak flour	11.5	24	14.2	1.226-1.475	4.1

**Table 2.** Mean±SD of flour chemical parameters by years.

Parameter	2019	2020	2021	2022	Total
<b>pH</b>					
Barbari flour	6.18 ± 0.22	6.10 ± 0.30	6.05 ± 0.21	6.09 ± 0.19	6.08 ± 0.25
Lavash flour	6.07 ± 0.28	6.92 ± 0.27	5.99 ± 0.32	6.06 ± 0.21	6.01 ± 0.29
Taftoon flour	6.02 ± 0.20	6.07 ± 0.31	5.84 ± 0.19	6.03 ± 0.34	6.02 ± 0.27
Sangak flour	6.11 ± 0.34	6.26 ± 0.22	5.98 ± 0.27	6.20 ± 0.36	6.13 ± 0.31
<b>Protein (g/100 g)</b>					
Barbari flour	13.14 ± 0.3.1	14.16 ± 4.30	13.16 ± 4.19	13.05 ± 1.72	12.80 ± 2.55
Lavash flour	14.00 ± 3.00	14.00 ± 3.00	13.70 ± 2.83	11.94 ± 0.99	13.56 ± 3.55
Taftoon flour	15.07 ± 3.71	13.78 ± 3.35	14.81 ± 5.74	18.48 ± 9.06	15.06 ± 5.12
Sangak flour	11.94 ± 1.03	12.62 ± 3.63	12.91 ± 2.32	14.77 ± 4.17	13.33 ± 3.30

Gluten (g/100 g)	Barbari flour	27.23 ± 3.31	30.60 ± 6.09	30.76 ± 4.68	29.27 ± 3.93	30.29 ± 5.12
Moisture (%)	Barbari flour	11.32 ± 1.31	12.57 ± 1.74	11.57 ± 1.20	11.79 ± 1.48	12.10 ± 1.54
	Lavash flour	32.38 ± 6.82	32.64 ± 4.25	30.41 ± 6.91	28.43 ± 2.83	30.59 ± 6.82
	Taftoon flour	30.25 ± 7.38	32.92 ± 4.86	27.00 ± 1.59	31.18 ± 4.72	31.07 ± 5.56
	Sangak flour	29.42 ± 5.12	29.09 ± 4.19	29.45 ± 6.59	26.84 ± 7.41	28.54 ± 6.14
Ash (g/100 g)	Barbari flour	1.03 ± 0.31	1.05 ± 0.16	1.08 ± 0.26	1.10 ± 0.20	1.07 ± 0.21
	Lavash flour	1.04 ± 0.23	1.11 ± 0.25	1.09 ± 0.20	1.00 ± 0.29	1.07 ± 0.22
	Taftoon flour	1.04 ± 0.23	1.04 ± 0.24	1.05 ± 0.11	1.06 ± 0.35	1.04 ± 0.23
	Sangak flour	1.08 ± 0.17	1.19 ± 0.31	1.08 ± 0.13	1.29 ± 0.28	1.17 ± 0.24

Table 3. Comparison between sample flour microbial parameters with national standard limit.

Parameter	2019	2020	2021	2022	Total
<b>Mold (%)</b>					
Barbari flour	0	5.88	0	14.28	2.70
Lavash flour	3.33	5.26	10	0	5.0
Taftoon flour	0	5.55	0	0	1.81
Sangak flour	12.5	0	0	0	3.84
<b>Total microorganisms (%)</b>					
Barbari flour	3.93	0	0	0	4.05
Lavash flour	0	0	0	0	0
Taftoon flour	0	5.55	0	0	1.81
Sangak flour	0	16.66	0	0	0

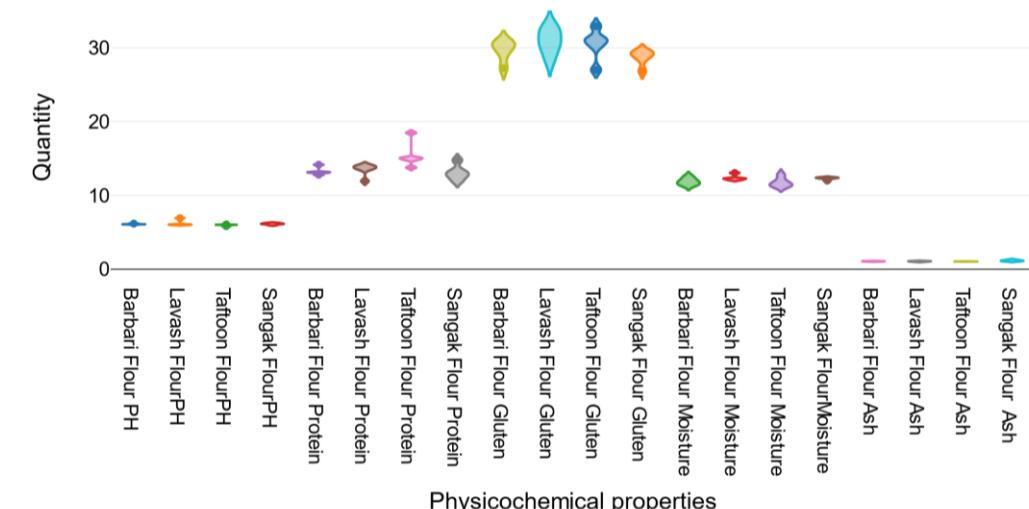
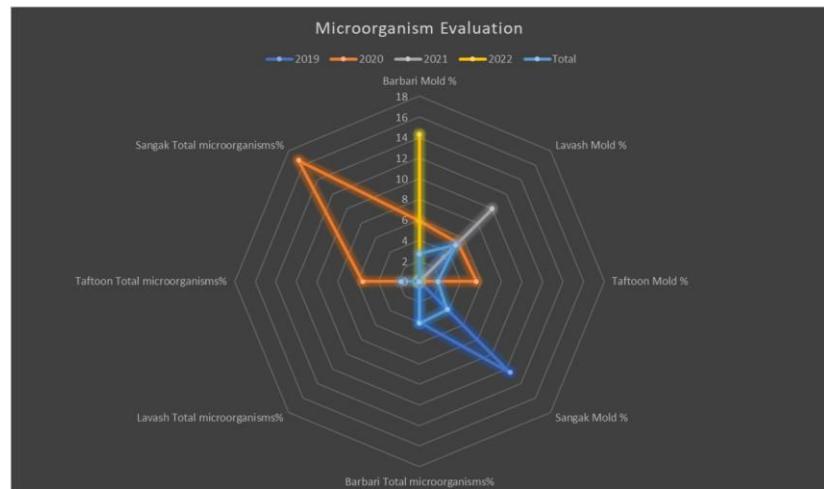
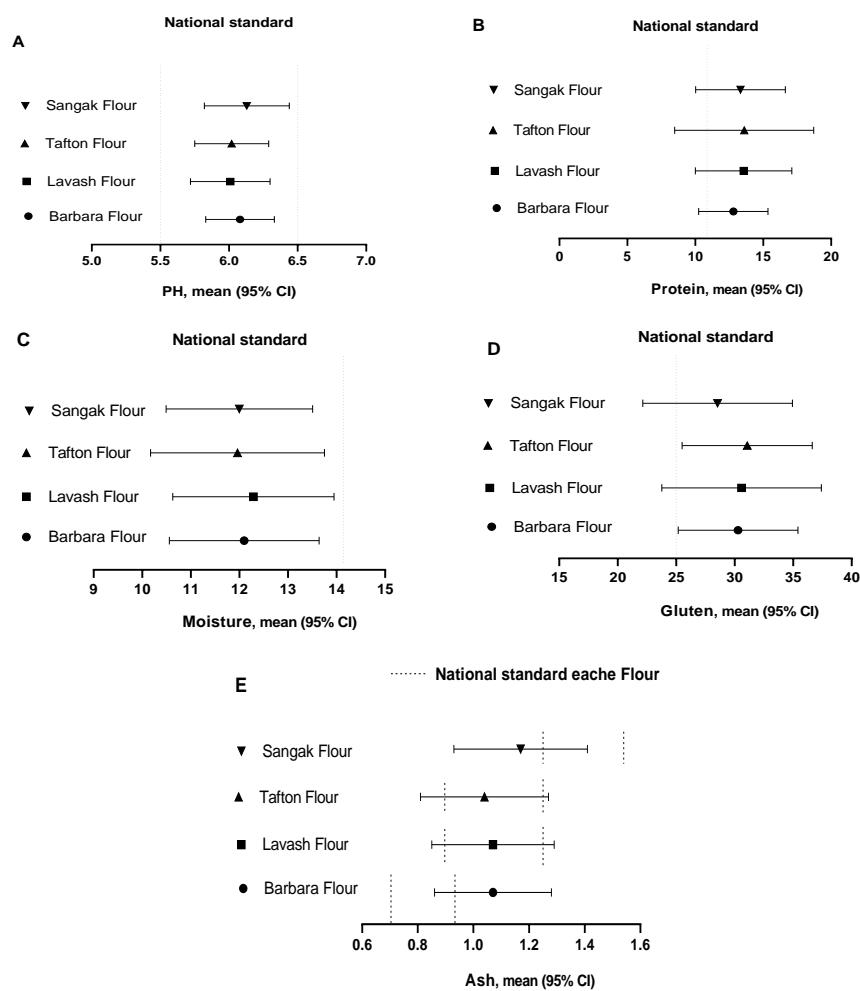


Figure 1. Violin plot of physicochemical properties.



**Figure 2.** Microorganism and mold evaluation



**Figure 3.** Comparing total sample flour chemical parameters regarding mean value with the national standard limit in 2019 -2022.

## Discussion

According to the results of the present study, the pH level in all four flour samples was in the range reported by national standards (between 5.6 and 6.5). Since pH level and acidity indicate fresh or aged flour, the results of this study presented the freshness of flour samples and their ideal storage conditions (Bahrami and Shahedi, 2004, Schuler *et al.*, 1995).

The average protein content in all the flour samples was higher than the standard limit regarding protein content (11%). The flour used to make Iranian Sangak bread in 2019, and Taftoon bread in 2021 had the lowest and highest amounts of protein, which could depend on the genetic content of cultivated wheat, weather conditions, and methods of bad cultivation conditions (Nasir *et al.*, 2004). Wheat flour's protein content (10-16%) significantly affects the texture and taste of baked products, with higher levels advantageous for commercial baking due to improved gluten development. Gluten creates a viscous, elastic structure that imparts essential physical qualities like plasticity, viscosity, and elasticity to the dough (Kaminski *et al.*, 2011). Having enough gluten content allows the dough to absorb water effectively. The strength and quality of gluten play a critical role in enhancing water absorption and dough elasticity, proving beneficial for carbon dioxide retention throughout the fermentation process in bakery and pastry items (Hădărugă *et al.*, 2016). In agreement with the present study, Aydin *et al.* and Ekinci *et al.* reported protein levels higher than their acceptable level at (7-13.5) (Aydin *et al.*, 2009, Ekinci and Unal, 2003). In other studies, Puppo *et al.* and Baljeet *et al.* reported wheat flour protein at 10.9% and 13%, respectively (Baljeet *et al.*, 2010, Puppo *et al.*, 2005).

Moisture is an essential parameter in different types of flour, which is directly related to the growth of microorganisms and the shelf life of flour (Hădărugă *et al.*, 2016), while it is particularly significant for achieving the desired qualities in bread, including texture, flavor, volume, and shelf life. Therefore, maintaining low

moisture levels is advantageous for extending the shelf life of the product (Schalk *et al.*, 2017). According to Iran's national standard, the moisture limit for different types of flour is 14.2%. In the present study, like Sadeghi Dehkordi Z *et al.* and Akpe *et al.*, the moisture content of all wheat flour samples ranged between 13.04-11.22, which was significantly lower than the standard value (Akpe *et al.*, 2010, Sadeghi Dehkordi *et al.*, 2017). Wheat flour typically has a moisture content that is too low to support the growth of most microorganisms; however, even small fluctuations in the moisture content of the flour can create conditions favorable for the development and propagation of molds and toxins (Aydin *et al.*, 2009).

Generally, the ash content of composite bread samples increases as the level of supplementation increases. The values obtained from the ash content of all flour samples were between 1 and 1.29, which were in the standard range. The ash content of flour significantly impacts baking performance and characteristics of bread. Higher ash content can enhance bread's nutritional value and flavor but may also result in a denser texture and a shorter shelf life due to increased fermentation activity and potential for greater water retention (Marshall, 2010). An acceptable amount of ash can prevent the turbidity of the bread and improve its quality (Dziki and Laskowski, 2005). The amount of ash in previous studies was varied. In the study by Oppong *et al.*, the amount of ash in wheat flour in Belgium was about 1%. Furthermore, Baljeet *et al.* and Saeid *et al.* reported it at 1.32% and 0.387-0.707%, respectively (Baljeet *et al.*, 2010, David *et al.*, 2015, Saeid *et al.*, 2015). This difference in the wheat flour ash could be related to how wheat is ground and processed and the amount of bran in the flour (Cardoso *et al.*, 2019, Ekinci and Unal, 2003).

The amount of gluten in all samples was higher than the standard level that the FDA set, which can improve the flour's physical and rheological properties and the proper absorption of water by the dough (Hădărugă *et al.*, 2016). On the other hand, it helps get appropriate volume and porosity

in wheat products and prevents early bread staling (Bahrami and Shahedi, 2004, Kaminski *et al.*, 2011). Gluten is a complex compound primarily composed of two types of proteins: gliadins and glutenins. Gliadins are soluble in ethanol and are characterized as prolamins, while glutenins, which are glutelins, are soluble in weak acid solutions. Both proteins exhibit high genetic polymorphism, with their proportions and types varying based on the genetic traits and environmental conditions of the grains. Gliadins are mostly monomers, while glutenins form polymers that are linked by disulfide bonds, categorized into high molecular weight (HMW) and low molecular weight (LMW) subunits. These disulfide bonds are crucial for maintaining the proteins' structure, as they form post-synthesis and contribute to the characteristic folding of gluten proteins. Both gliadins and glutenins play significant roles in the functional and rheological properties of wheat and other gluten-containing grains (Schalk *et al.*, 2017). Regarding this fact, the Iranian Taftoon flour sample had the highest total gluten. In a study by Cardoso *et al.* in Portugal, gluten content was reported to be 25 % (Cardoso *et al.*, 2019). Frakolaki *et al.* in Greece also reported a gluten content of wheat flour of 28.24% (Frakolaki *et al.*, 2018). Besides the positive effects of gluten on the quality of bread, its excess amount can negatively affect people with Celiac Disease (Ortolan and Steel, 2017).

The flour microflora comprises molds, yeasts, mesophilic bacteria, and other bacteria (Aydin *et al.*, 2009). This study showed that the amount of TVC in 3 samples of Iranian Barbary flour, Taftoon, and Sangak flour between 2019-2022 had different values, among which the highest contamination was related to Sangak flour in 2020 with 16.66%. The results of this study were in agreement with Eglezos' study, who reported the overall count of microorganisms  $4.2\text{LOG}_{10}$  CFU/g (Eglezos, 2010). In another study in North America by Manthey *et al.*, the TVC value ranged from 0.9 to 8.4 CFU/g (Manthey *et al.*, 2004). Furthermore, Berghofer *et al.* reported the TVC of Australian wheat flour at 5.0 CFU/g (Berghofer *et*

*al.*, 2003).

This contamination of wheat flour may occur during the harvesting, processing, and storage of wheat grains. Microorganisms are constant contaminants of wheat flour because they have existed in wheat since it started growing (Plavšić *et al.*, 2017). However, flour is a non-perishable food and cannot be potentially a foodborne illness. This is because the flour's low moisture and water activity do not allow pathogenic bacteria to grow and propagate (Berghofer *et al.*, 2003).

According to the results, mold contamination was present in all four flour samples. Iranian Lavash flour, with 5%, and Iranian Taftoon flour, with 1.8%, had the highest and lowest contamination, respectively. The following researchers reported similar results. Aydin *et al.* and Cardoso *et al.* in Turkey and Portugal reported that the rate of mold contamination was roughly 10 CFU/g (Aydin *et al.*, 2009, Cardoso *et al.*, 2019). Contamination of flour samples with molds was also reported by Eglezos, Sadeghi Dehkordi *et al.*, and Rezazadeh *et al.* (Eglezos, 2010, Rezazadeh *et al.*, 2013, Sadeghi Dehkordi *et al.*, 2017). Mold contamination is related to several factors, including high temperatures, contamination of bakeries, flour storage, or grain storage silos, as well as contamination during production, insufficient cleaning of wheat mills, and increased atmospheric humidity (Rezazadeh *et al.*, 2013). Molds in a humidity of 15% propagate quickly and can produce mycotoxins (Eglezos, 2010). There is evidence that mycotoxins produced by three types of fungi, Aspergillus, Penicillium, and Fusarium, can be absorbed into body and lead to mycotoxicosis (Sadeghi Dehkordi *et al.*, 2017). Additionally, if most molds grow in the flour products mycotoxin, the flour will be whole of mycotoxins that cannot be removed. Therefore, using these flours and the consumption of resulting bread may lead to dangerous diseases such as cancer and can seriously threaten people's health (Rezazadeh *et al.*, 2013).

### Conclusion

The results of this study revealed all

physicochemical parameters of flour (pH, moisture, ash, gluten, and protein) were according to the Iran National Standards Organization, which shows the proper quality of the flour. It can meet consumers' expectations of high-quality products. However, the quality of wheat flour and bread produced can be improved by adding other nutritious products. In the analysis of microbial properties, bacterial contamination of the flour was observed. However, contamination of all flour samples with mold was a matter of concern, which carries the risk of increasing mycotoxins and causing dangerous diseases in the consumer. Therefore, it is necessary to take measures such as proper transportation and storage of the flour, control and prevent moisture changes in wheat and flour storage warehouses, as well as accurate and hygienic cleaning of wheat and flour processing machines and reducing their microbial load to ensure the safety of the product.

### Authors' contribution

All the authors were involved in designing, data gathering, statistical analysis, and writing the original draft of the manuscript and approved the final manuscript.

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### Conflict of interest

The authors declared no conflict of interests.

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