

Quality of Flour Types, in the Bakeries of Hamedan, Iran during 2015-2016

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Background & Aims of the Study: Wheat flour makes the basic component of bread. Thus, evaluating its quality plays an important role in public health. This paper focused on the physicochemical and microbial analysis of all bread flour types, used in the bakeries of Hamedan, Iran during 2015-2016.

Materials & Methods: All bread flour types such as Barbari, Lavash, Sangak and Tafton bread flours were collected from 100 bakeries which baked various types of wheat bread in Hamedan, western Iran from May 2015 to February 2016 in this cross-sectional study. Moisture, dry matter, ash, fat and protein percent values of samples were analyzed. Total viable counts of bacteria as well as yeasts and molds of all samples were determined. Identification and characterization of enumerated fungi was also identified.

Results: The highest moisture content percent belonged to Sangak flour (12.75 ± 0.06), significantly ($P < 0.05$) followed by Taftoon (12.57 ± 0.03), Barbari (11.77 ± 0.03) and Lavash (11.57 ± 0.03), respectively. The order of ash percent was as follows: Lavash (1.36 ± 0.01), Sangak (1.11 ± 0.04), Barbari (1.03 ± 0.03) and Taftoon (0.93 ± 0.02). According to the results, Taftoon flour meaningfully ($P < 0.05$) had the highest crude protein value ($5.31 \pm 0.17\%$), followed by Sangak ($4.11 \pm 0.03\%$), Barbari ($2.75 \pm 0.05\%$) and Lavash ($2.40 \pm 0.09\%$), respectively. Protein and fat deficiency were observed in all flours. Total viable counts of bacteria were in permissible range in all flours. Yeasts and molds of all samples exceeded recommended limits.

Conclusion: It was found that the fungal growth control is essential by improving storage conditions, hygienic operations and nutrients enrichment in wheat flours of all bread types of Hamedan, Iran.

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Background

Bread is specifically important as an important nutritional source across the globe, supplying some of our daily energy requirements, proteins, minerals and B vitamins. Iranians receive about 60-65% of their protein and calorie as well as 2-3g of their daily minerals from bread (1,2). Bread is one of the frequently-consumed foods in most countries. Its quality evaluation has an important role in public health (3-5) for its high daily

consumption per capita (314-505 g per day). Wheat flour which makes the main ingredient of bread is a powder made of grinding wheat, used for human consumption.

Generally, food quality evaluation is conducted by microbial and chemical analysis. Wheat flours' quality can be determined by various characteristics such as crude protein, moisture, ash and fat. Upper protein percent (10-14.5%) indicates that the potent and intense the flour and lesser protein value (6-10%) will lead to the smooth flour. For producing hard and chewable

bread, full protein flour is applied; but, for manufacturing bakery product such as cookie, biscuit and cake, low protein flour is used. It has been generally determined that increased whole protein percent of the flour is for the enhancement of gluten value (6). Moisture content is regarded as an important factor in characterizing the durability and quality of the flour. Normal moisture content in wheat flour is 15.5%. If increased moisture content be higher than natural-limit flour becomes sensitive to fungi especially mold increase because of higher enzyme operation, insect invasion and sensorial changes are likely. High moisture value of flours can be lead to high lipolysis and proteolytic enzyme operations which further may ultimate to the damage of vital nutrients such as lipid and protein (7). The ash value is a parameter of whole content of minerals in wheat flour. Ash percent demonstrates milling efficiency, indicating the amount of bran pollution indirectly in flour (8,9). Mineral value also is affected by different characteristics such as soil, climate, variety and cultural features. Ash, the content of flour, can change the color of produced products to a darker color. The white bakery products should make by wheat flour containing low ash value; while whole wheat flour has high ash percent (9).

Microbial food pollution and their influences on human body are very important issues (4). Unawareness of farmers and food handlers about the growth conditions and effective factors of microorganisms and fungi may induce considerable economic issues and health problems (5,10). Temperature, moisture, sunlight intensity, air and soil pollution; also, general conditions of the seeds as environmental factors are important causes in cereals 'spoilage' (11).

The fungi are the main factors in bakery products and breads deterioration which may result due to lower humidity of them compared to other foods. Fungal contamination of wheat and flour induces harmful effects on the flour quality, leading to proteolysis, lipolysis and

saccharolysis effects; it may also produce aroma-reducing compounds and decrease gluten level as well as rheological properties of dough (10). In addition, some fungi produce secondary metabolites, named mycotoxins. Mycotoxins, the derivatives of acetate or amino acids that are mostly produced by *Aspergillus*, *Penicillium*, *Fusarium*, *Claviceps* and *Alternaria* genera (11). According to the news of Food and Agriculture Organization (FAO), about 25% of globally-produced food materials are polluted by mycotoxins.

Being usually heat resistant, mycotoxins are not destroyed due to the baking heat. Continual consumption of contaminated food with mycotoxins raises the likelihood of the respiratory, digestive and nervous problems (8). One big concern about permanent use of mycotoxins along with foods is increased risk of carcinogenesis, mutagenesis and teratogenesis in consumers (10,11). Improper storage conditions during grain ripening can induce mold growth and then mycotoxin production.

Mycotoxin pollution is propagated, undestroyed and condensed in produced flour as wheat seeds are milled into flour; thus, wheat flour may have significant mycotoxigenic contamination. The wheat seeds are likely to be contaminated with different microorganisms like bacteria and fungi during wheat production stages such as harvest, storage and transport, inducing toxin production from which they contaminate the flour while milling (10).

Aims of the study:

This study conducted physicochemical and microbial analysis of all bread flour types, used in the bakeries of Hamedan, Iran during 2015-2016, regarding the significance of wheat in globally-consumed food especially Iranian food.

Materials & Methods

Sample collection

Collected samples included Lavash, Barbari, Sangak and Taftonbread flours. Collected flour

samples from 100 bakeries in 10 areas of Hamedan are represented in Table (1).

Table 1) Sampling regions

| Region | Lavash | Barbari | Sangak | Taftoon |
|-------------------|--------|------------|--------|---------|
| Modares avenue | 3 | 1 | 4 | 2 |
| Ostadan | 4 | 3 | 3 | 1 |
| Babataher | 5 | 2 | 4 | 2 |
| Alvand | 2 | 1 | 4 | - |
| Ekbatan | 4 | 2 | 3 | 1 |
| Kermanshah avenue | 3 | 1 | 3 | - |
| Abbasabad | 4 | 2 | 4 | 1 |
| Hegmatane | 4 | 2 | 3 | 1 |
| Shariati avenue | 4 | 2 | 3 | 2 |
| Zamani | 5 | 1 | 3 | 1 |
| Total | | 100 | | |

Flour samples were collected from 100 bakeries which baked different types of wheat bread. Three gunny-bags were randomly selected in each bakery and using a special device for flour sampling, flour samples were collected from the middle of each gunny-bag. Collected in sterile polyethylene bags, the samples were quickly brought to the Lab of Department of Food Hygiene and Quality Control in Bu-Ali Sina University.

Proximate analysis of samples

According to the manual of Association of Official Analytical Chemists (AOAC) on dry matter basis, the bread flours were analyzed for moisture, ash, crude protein and lipid (ether extract) or crude fat by drying, dry ashing, Kjeldahl and Soxhlet procedures, respectively (12).

Bacterial analysis

Bread flour (25g) was diluted and homogenous by 225ml of sterile peptone water 0.1% (Merck, Darmstadt, Germany) in a stomacher apparatus for 1 min. For enumerating of total viable count (TVC), serial dilutions of samples were prepared and 0.1 ml of them was spread on the surface of Plate Count Agar (PCA, Merck, Darmstadt, Germany) plates. The plates were incubated for 2 days at 30 °C. Depending on the contamination level, four dilutions in three replicates of flour homogenates were cultured.

Enumerated colony forming units (CFU/g) were converted into logarithms values.

Identification and characterization of fungi Fungi isolation from bread flour was conducted based on the study of Samson et al (13). Under sterile conditions, all analyses were done in triplicate. About 25g of each sample was diluted and homogenous by 225 ml of sterile peptone water 0.1% (Merck, Darmstadt, Germany) in a stomacher apparatus for 1 min. For fungi enumeration, PDA, DRBC (Dichloran Rosebengal and Chloramphenicol Agar) and DG18 (Dichloran Glycerol 18% Agar) mediums were prepared. Surface culture or pour plate method was used for sample culturing on the plates. Incubation time was considered 7 days at 28°C. After this period, the plates were evaluated for growth of yeast and molds. Enumerated colony forming units (CFU/g) were converted into logarithms values. First, all fungal species were isolated into Czapek Yeast extract agar (CYA) and malt extract agar (MEA) plates for future detections and incubated for 7 days at 25°C. Then, isolated fungi, was determined regarding their macro and micro morphological features, using standard classification keys (14-16).

Data analysis:

All conducted analyses were performed triplicate in the present study. The calculated data are represented as mean values \pm standard deviation (SD). All enumerated colony forming units (CFU/g) were converted into logarithms values. The statistical analysis of obtained data was conducted, using SPSS (IBM SPSS statistics 21). To compare the differences of mean values, Tukey's test was used at the significance level at $P < 0.05$. Also, comparing obtained data with standard values was done by one- sample t-test method.

Results

Proximate sample analysis

The content of moisture, ash, protein and fat of all flour samples are presented in Table 2.

Table 2) Physicochemical parameters of wheat flour of bread types

| sample | Moisture (%) | Ash (%) | Crud protein (%) | Lipid (%) |
|----------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Lavash | 11.57±0.03 ^d | 1.36±0.01 ^a | 2.40±0.09 ^c | 1.13±0.007 ^b |
| Barbari | 11.77±0.03 ^c | 1.03±0.03 ^{bc} | 2.75±0.05 ^{bc} | 1.13±0.005 ^b |
| Sangak | 12.75±0.06 ^a | 1.11±0.04 ^b | 4.11±0.03 ^{ab} | 2.28±0.003 ^a |
| Taftoon | 12.57±0.03 ^b | 0.93±0.02 ^c | 5.31±0.17 ^a | 2.27±0.009 ^a |
| Standard limit | 15.5 | 3 | 7-8 | 3 |

Table 3) Qualitative variations of fungi in wheat flour of bread type

| Sample | <i>A. fumigatus</i> | <i>A. flavus</i> | Penicillium | Mucor | Cladosporium | Rhizopus | Ulocladium |
|---------|---------------------|------------------|-------------|-------|--------------|----------|------------|
| Lavash | - | + | - | - | - | + | - |
| Barbari | - | - | + | - | + | - | - |
| Sangak | + | + | + | + | - | - | - |
| Taftoon | - | + | + | + | + | + | + |

Moisture percent of samples was in the range of 11.57-12.75. The highest moisture content percent was observed in Sangak flour (12.75±0.06) significant at (P<0.05), followed by Taftoon (12.57±0.03), Barbari (11.77±0.03) and Lavash (11.57±0.03), respectively. The order of ash percent in the samples was as follows: Lavash (1.36±0.01), Sangak (1.11±0.04), Barbari (1.03±0.03) and Taftoon (0.93±0.02).

The highest fat contents of 2.28±0.003 and 2.27±0.009% which were significantly (P<0.05) belonged to Sangak and Taftoon flours; but, the lowest fat levels of 1.13±0.005 and 1.13±0.007% were observed in Barbari and Lavash bread flours. Based on the results, Taftoon flour had the highest crude protein content (5.31±0.17%) significant at P<0.05 among all of the samples, followed by Sangak (4.11±0.03%), Barbari (2.75±0.05%) and Lavash (2.40±0.09%), respectively.

Microbiological analysis

A total count of bacteria and yeasts-molds of samples are represented in Figs.1 and 2.

The highest bacteria count (4.87 log CFU/g) was obtained in Sangak and Taftoon, followed by Lavash (4.05 log CFU/g) and Barbari (3.7 log CFU/g) flours, respectively.

Similar to the bacterial results, Taftoon (log 4.98 CFU/g) and Sangak (log 4.87 CFU/g) flours had the highest amounts of yeast and molds, followed by Barbari (log 3.72 CFU/g) and Lavash (log 3.31 CFU/g), respectively.

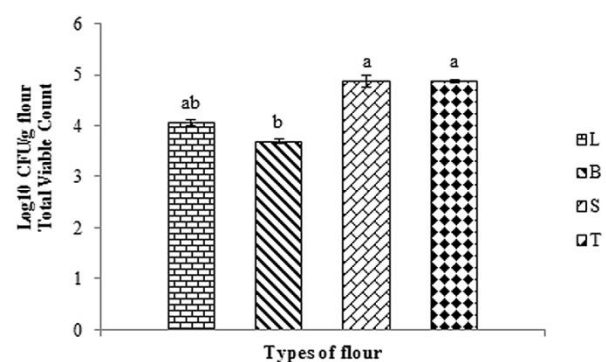


Figure 1) Total counts of bacterial pollution in wheat flour of bread types. Significant differences (P<0.05) among columns were shown by different letters

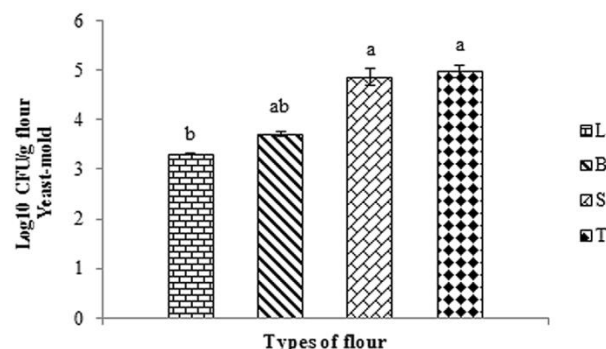


Figure 2) Total counts of fungal pollution in wheat flour of bread types. Significant differences (P<0.05) among columns were shown by different letters

Results also showed that fungal contamination of wheat flour was considerable and the isolated fungi frequency was as follows: Penicillium spp. (28%), Aspergillus flavus (16%), Aspergillus fumigatus (16%), Cladosporium spp. (12%), Rhizopus spp. (12%), Mucor spp. (8%) and Ulocladium spp. (8%) (Fig.3).

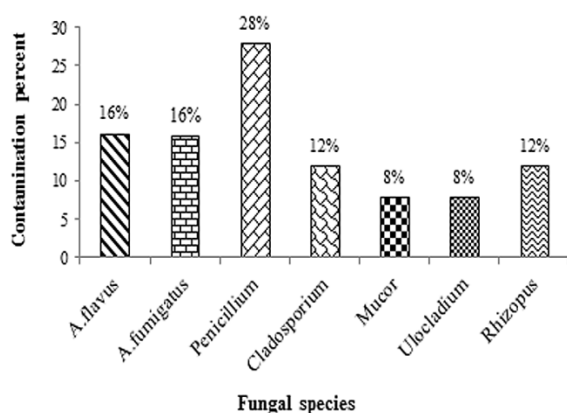


Figure 3) Percent of various fungi contamination in wheat flour of bread types

Based on Table 3, Taftoon flour contains a mixture of various fungi, including: *A. flavus*, *Penicillium* spp., *Mucor* spp., *Cladosporium* spp., *Rhizopus* spp. and *Ulocladium* spp. *A. fumigatus*, *A. flavus*, *Penicillium* spp. and *Mucor* spp. were also observed in the Sangak flour. *A. flavus*, *Rhizopus* spp., *Penicillium* spp. and *Cladosporium* spp. were isolated from Lavash and Barbari flours, respectively.

Discussion

Wheat is a major food product, broadly consumed across the globe. This study tried to determine different quality parameters and microbial contaminations of all bread flour types in Hamedan. Based on WFP (2012), moisture and ash standard values of wheat flour should not be higher than 15.5 and 3%, respectively (17). In this study, moisture and ash value of all flours were significantly ($P<0.05$) lower than standard values. Results of this study indicated that the fat percent of all flours were significantly ($P<0.05$) lower than standard value (3%). In their studies on wheat flour, Akpe *et al.* (2010), Aydin *et al.* (2009) and Batool *et al.* (2012) obtained moisture, ash and fat contents at the range of 9-13, 2-3 and 1.5-2.5% (18-20). In case of the flour quality and acceptability of flour products, moisture is an important factor, affecting the shelf life and microbial growth (21).

The minimum content of protein in wheat meal flour is 7-8% for determining proper quality of the final product of wheat flour. Considering the nutritional value of the wheat and the stability of the flour product, it is a very important parameter (22). Results of this study disagree with Aydin *et al.* (2009), Ekinic and Unal (2003), with protein contents of wheat flour ranging from 7-13.5% (19,23). Other authors indicated that wheat flour protein was 10.32%-11.58%. Their value was higher than our result (8). In Bangladesh, moisture, protein, fat and ash percent of wheat flour were 9.90-12.48%, 8.67-12.47%, 0.893-1.387% and 0.387-0.707%, respectively (6). In another study of Baljeet *et al.*, fat, moisture, ash and protein contents of refined wheat flour were 1.78%, 13.29%, 1.32% and 13%, respectively (9). The difference of moisture, fat and other ingredients in different brands of wheat flour is closely correlated to the genetic structure of wheat varieties, agricultural and the weather conditions (7).

However, all bread flour types represented significantly lower protein content ($P<0.05$) than the identified standard. Low moisture, protein and fat content in the samples of this study may be attributed to the long-term storage of wheat in the stores that can lead to the dehydration, proteolysis, lipolysis and oxidation of flour proteins and fats. Low ash value in wheat flour of all breads can be linked to mineral deficiency in wheat seeds due to the mineral deficiency in farmland soils (18-20).

The maximum legal limit for bacteria in flour is 5log CFU/g. Thus, all of our samples were in the allowed range (17). Manthey *et al.* (2004) found the aerobic plate counts of durum wheat in North America ranging from 0.9 to 8.4log CFU/g (24). Berghofer *et al.* (2003) represented that the Total Viable Counts in the Australian wheat for aerobic mesophilic bacteria was 5.0 log CFU/g (25). However, the flour is known as a non-perishable food that can be correlated to its low humidity and followed water activity

characteristic; thus, bacteria cannot simply grow in it (25).

The maximum legal limit for fungi in flour is 3log CFU/g (17). Yeasts and molds of all samples exceeded the recommended values. Other authors obtained similar results in case of microbial contamination in flour. They reported that the predominant and frequently- isolated wheat flour fungus was *Aspergillus niger* (48.4%) (18,20,26). Isolating *Aspergillus* spp. (33%) and *Penicillium* spp. (25%) from the wheat flours in one study, Ntuli *et al.* (2013) represented that yeasts and molds from the wheat flours were within the recommended limits(27). Their result disagrees with this study.

In another study, fungal contamination of wheat flour was found to be considerable (31.5%) and major fungal genera were *Aspergillus* spp. (especially *A.niger* and *A. fumigatus* spp.), *Fusarium* spp. and *Acremonium* spp. (28). They concluded that improper conditions like high temperature, contamination of the bakeries' flour stocks or silos of industries, microbial spoilage of seeds or contamination during production process are likely to affect increased fungal contamination of wheat flour; those factors may finally cause producing mycotoxins.

Other scholars suggested that high isolation frequency of *Aspergillus* spp. and *Penicillium* spp. from the flour samples can be linked to poor handling of the raw materials in the food chain. Growing well into the grains and producing toxins, storage molds like *Aspergillus* spp., *Penicillium* spp., and the soil molds such as *Fusarium*spp can be impossible to remove during food processing. Thus, destroying microscopic fungi mycelium does not lead to deconstructing produced mycotoxins in food products (29). In addition to standard-level, fungi deteriorates the food quality and enhancing the risk of serious food-borne diseases. Fungal food contamination can cause the food ingredients' decomposition. Many food fungi can produce mycotoxins (30).

Used imported wheat varieties for baking bread were analyzed for the fungal contamination in another study. Results indicated the existence of pathogenic fungi including *Ulocladium*, *Cladosporium*, *Alternaria*, *Rhizopusnigricans*, *Penicillium*, *Trichothecium*, *Mucor*, *Penicillium* and *Aspergillus* species (31). Another study indicated *Aspergillus* and *Fusarium* species in flour samples in India (32); Schollenberger *et al.* (1999) reported the major fungal contaminations of wheat flour to be *Aspergillus* species as well (33). In another paper, *Aspergillus*, *Penicillium*, *Cladosporium* and *Eurotium* species were the most common isolated molds of Australian wheat (25).

Aspergillus, *Penicillium*and *Fusarium* contamination in food can lead to mycotoxin production that cause mycotoxicoses after their absorption in the consumer bodies (34). The mycotoxins mostly are mutagenic, carcinogenic and teratogenic; they can lead to toxicity in various organs of body. Some species of yeasts and molds induce various infections and diseases in consumers (30).

Conclusion

Regarding the fact that wheat is the major common food worldwide, following and noticing all steps of production, distribution and maintenance of wheat and flour has specific significance. According to the results in this study, lower content of the moisture, ash, fat and protein compared to the standard values in wheat flour of all bread types in Hamedan shows improper storage conditions of wheat, leading to the nutrients' destruction in wheat flour. Essential operations for bread quality elevation are improving wheat storage conditions and enrichment of wheat flours with other nutritious products. Also, the microbiological analysis showed that fungal contamination of all samples was concerning in contrast to the bacterial population. These detected molds are known to produce toxins that can lead to acute and chronic toxicity in

various consumers such as animals and humans. Thus, the need to controlling fungal growth in bread and bakery products, using hygienic operations and preservatives, seems essential.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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