Evaluating Microbial Contamination of Bakeries, Wheat Flour in Ghaemshahr, Iran

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Recommended Citation
(Received: Jun 24, 2023; Accepted: Oct 16, 2023; Published: Dec 27, 2023)

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EVALUATING MICROBIAL CONTAMINATION OF BAKERIES, WHEAT FLOUR IN GHAEMSHAHR, IRAN

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ABSTRACT

Although flour is regarded as a safe product from the perspective of microbiology due to its low water activity, it can be a suitable environment for the growth of microorganisms if suitable conditions are provided for their growth and reproduction. The objective of this study was to examine the microbial quality of 82 % and 78 % flours distributed in 5 bakeries in the city of Ghaemshahr. The evaluation of the results of the physicochemical tests on the samples revealed that sample No.5's moisture content (82 % of the flour sample from the bakery E) was significantly higher than other samples, while samples No.2 and No.9 had the lowest moisture levels (82 % of the flour sample from the bakery B and 78 % of the flour sample from the bakery D, respectively; all of the samples' moisture contents were within the standard range. All of the flour samples had microbial contamination, according to the evaluation of the results of the microbial tests performed on them, but sample No.1's (82 % of the flour sample from bakery A) microbial population and sample No.8's mold population were significantly higher than those of the other samples. On the other hand, lower mold contamination was seen in flours with a higher level of extraction. There was no statistically significant difference between the mentioned treatments, and the yeast population was significantly higher in samples No.1 (82 % of the flour sample from bakery A) and No.7 (78 % of the flour sample from bakery B) than in the other samples.

Keywords: Flour, mold, total microbial population, yeast.

INTRODUCTION

About 19 % of the world's caloric resources come from wheat, a product that is grown in 120 different countries. About 529 trillion grams of dry wheat are produced worldwide each year. In the last three seasons (2018–2021), the global wheat acreage was 213.9–219.0 million ha and grain yields ranged from 732.1 to 760.9 million tones. The largest global wheat producers in the 2020–2021 were China (134.3 million ton). China is the world's largest producer of wheat, accounting for about 18 % of global production (Mitura et al., 2023). Protein, calories, plant chemical compounds, antioxidants, and bioactive compounds that enhance and promote health can be found in wheat (Gupta et al., 2021). More than 50 % of the world's total caloric intake comes from wheat (Triticum aestivum L.) flour, which is used to make baked products like bread and biscuits (Masood et al., 2020). According to Senya et al. (2002), whole grains are high in nutrients like protein (8–16 %), fat (1-3 %), and fiber (12–15 %). It lacks lysine and threonine but is abundant in methionine and cysteine (Peluola-Adeyem et al., 2021). The primary ingredient in bakery products is wheat flour. It has a lot of starch and other substances like proteins and lipids, which have an impact on its properties (Panasiti et al., 2021).

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livestock. Wheat is regarded as a food that is microbiologically healthy because of its lower water content. Despite the low moisture content of wheat, research has shown that microorganisms can persist in an inactive state for a long time before emerging when they find a more favorable environment (Feng and Archila-Godínez, 2021). As would be expected, the main wheat grain and the microbial quality of wheat flour are closely related (Berghofer et al., 2003). Because bran contains a high concentration of microbial contaminants, research has shown that milling has been significantly effective in reducing microbial contamination (Sabillón et al., 2016a, 2016b). The quality and safety of products can, however, be significantly impacted by certain microorganisms and molds that can persist in flour. Recent studies and reports indicate that there may be more and more severe flour contamination than previously thought. Flour has not been considered a source of pathogens historically, due to the inability of bacteria to grow in these products. However, pathogenic bacteria have been isolated from wheat flour samples and associated with flour and flour-product foodborne outbreaks and recalls. Consumer who handle flour improperly, such as those who consume homemade play dough and raw pastry dough, run the risk of compromising food safety (Feng and Archila-Godínez, 2021). According to studies, grain-based products are a common source of Salmonella and Escherichia coli (Forghani et al., 2019). Due to various sources of contamination of the wheat surface, wheat flour may have a high microbial load. This situation contributes to the spread of diseases brought on by wheat-based food products (Ari Akin et al., 2023). The seed head is exposed to a number of microbial contamination sources when it is taken out of the flag leaf sheath, including air, dust, water (rainfall and irrigation), and insects. As a result, the microflora of wheat grain may be extensive and diverse and comprise bacteria, yeasts, and molds. The pre-harvest, harvest, transportation, storage, and processing stages are just a few of the wheat production chain's potential points of microbial contamination (Los et al., 2018). Animals, air, water, dust, and contaminated materials can all carry contaminated microbes (Laca et al., 2006; Los et al., 2018). The type and amount of microbial load can also be influenced by other weather factors, including rainfall amount, relative humidity level, and particular farm microflora (Sabillón et al., 2016). Intestinal pathogens like Escherichia coli and salmonella, gram-positive bacteria like Bacillus cereus, yeasts, and mycotoxin-producing fungi from the Aspergillus, Penicillium, and Fusarium genera are among the microorganisms found on seeds (Laca et al., 2006).

Wheat grains typically have microbial load on their surface. However, microbial contamination is distributed differently among the mills during the dry milling process (Berghofer et al., 2003). This problem causes refined wheat flour's microbiological quality to be insufficient. Additionally, the risk of microbial contamination in whole-wheat flour and products made from it is common due to the rising trend of consumption of whole grain products. Although the dehydration activity of flour (aw < 0.60) does not support microbial growth, contamination spores and dormant microorganisms can persist for a long time and pose a risk to human health (Eglezos, 2010). It is possible to stop the spread of diseases brought on by contaminated wheat flour and food-borne disease when safety interventions are established based on consumer eating habits (Lopez and Simsek, 2020). This study looked into the microbial quality of flour from bakeries in Ghaemshahr, Iran. To this end, the microbial quality of the 82 % and 78 % flours distributed in 5 bakeries of Ghaemshahr, Iran were tested to determine the amount of mold and yeast.
contamination as well as the total microorganisms count.

MATERIALS AND METHODS

The aforementioned samples were taken from the city's bakeries and transported to the lab under the proper conditions of 4 degrees Celsius in order to assess the level of mold and yeast contamination of the flours distributed in 5 bakeries of Ghaemshahr, Iran, as well as to test the total microorganisms count. Included in the tested flours were 82% and 78% flours. A test for moisture content (Moisture determination test) was conducted by weighing 3 grams of flour, heating the container it was in to 130 degrees Celsius, and then weighing the sample once it had cooled. The moisture content of the sample is indicated by the weight difference, which represents the amount of moisture the sample has lost. In an electric oven, flour was heated to a temperature between 550 and 600 degrees Celsius in order to perform the ash determination test.

Formula 1:
\[
\text{Ash percentage} = \frac{\text{remained ash weight}}{\text{initial sample weight}}
\]

Mold and Yeast Test in Flour Samples

Initially, an autoclave was used to prepare and sterilize the Subro Dextrose Agar culture medium. Following, Ringer's solution was used to create serial dilutions from the tested samples. The colonies were counted and the average of two plates was calculated from the product of the number of colonies in the dilution series plates after the plates containing 1 ml of sample were added to the subro dextrose agar culture medium and shaken, warming in the incubator at 30 degrees Celsius for 72 hours (Fawole and Oso, 2001).

Total Microorganisms Count Test in Flour Samples

First, an autoclave was used to prepare and sterilize the Plate Count Agar culture medium. Following, Ringer's solution was used to create serial dilutions from the tested samples. The average of two plates was calculated from the product of the number of colonies in the dilution photo after the plate count agar culture medium was added to the plates containing 1 ml of sample and shaken, warming was applied in an incubator at 30 degrees Celsius for 72 hours, the colonies were counted, and the warming was applied (Fawole and Oso, 2001).

This study was carried out using a completely random design. The test was conducted at 5 bakeries in Ghaemshahr, Iran, and included 2 levels of flour type (78%, 82%). With this approach, the effects of various factors were evaluated, and the Duncan multiple range test was run to confirm the existence of mean differences. Data analysis was carried out using SPSS software's analysis of variance tables, mean comparison at a 95% confidence level, and Duncan test. All of the results were based on the average of three repetitions. Excel software was used to draw all of the tables and graphs.

RESULTS AND DISCUSSION

The results of the comparison of the mean moisture content of the samples are shown in Table 3 and Figure 1, and the variance analysis of the moisture of the samples is shown in Table 1. The samples' variance analysis revealed that the treatment had a significant impact on the moisture content of the flour samples (P < 0.05). The results of comparing the means of the samples revealed that sample 5's moisture content was significantly higher than that of the other samples (P < 0.05), while samples 2 and 9's moisture content was the lowest (P < 0.05). The maximum moisture content of 82% and 78% flours
is 14.2 % by weight, and all of the samples that were examined fell within this range, as per Iranian national standard No. 103.

Table 1: Variance analysis of moisture of flour samples

<table>
<thead>
<tr>
<th>Sources of changes</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Treatments</td>
<td>9</td>
<td>14.33</td>
<td>260.09</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Significant difference at the 5 % probability level

Table 2: Variance analysis of ash of flour samples

<table>
<thead>
<tr>
<th>Sources of changes</th>
<th>Degree of Freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between treatments</td>
<td>9</td>
<td>0.157</td>
<td>10.094</td>
<td>0.000 **</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Significant difference at the 5 % probability level

Table 3: Results of physicochemical tests of flour samples

<table>
<thead>
<tr>
<th>Test</th>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>code (1)</td>
<td>6.39±0.19 b</td>
<td>1.11±0.08 ab</td>
<td></td>
</tr>
<tr>
<td>code (2)</td>
<td>5.60±0.18 c</td>
<td>1.06±0.08 ab</td>
<td></td>
</tr>
<tr>
<td>code (3)</td>
<td>6.39±0.09 b</td>
<td>1.21±0.11 a</td>
<td></td>
</tr>
<tr>
<td>code (4)</td>
<td>6.58±0.14 b</td>
<td>1.00±0.04 abc</td>
<td></td>
</tr>
<tr>
<td>code (5)</td>
<td>13.07±0.07 a</td>
<td>0.69±0.18 def</td>
<td></td>
</tr>
<tr>
<td>code (6)</td>
<td>6.37±0.43 b</td>
<td>0.91±0.08 abcd</td>
<td></td>
</tr>
<tr>
<td>code (7)</td>
<td>6.42±0.28 b</td>
<td>0.82±0.20 cde</td>
<td></td>
</tr>
<tr>
<td>code (8)</td>
<td>6.37±0.32 b</td>
<td>0.71±0.15 def</td>
<td></td>
</tr>
<tr>
<td>code (9)</td>
<td>5.58±0.12 c</td>
<td>0.51±0.06 f</td>
<td></td>
</tr>
<tr>
<td>code (10)</td>
<td>6.65±0.23 b</td>
<td>0.63±0.12 ef</td>
<td></td>
</tr>
</tbody>
</table>

Different letters indicate significant differences (P ≤ 0.05).

Samples: code (1): 82 % flour sample from bakery A, code (2): 82 % flour sample from bakery B, code (3): 82 % flour sample from bakery C, code (4): 82 % flour sample from bakery D, Code (5): 82 % flour sample from bakery E, code (6): 78 % flour sample from bakery A, code (7): 78 % flour sample from bakery B, code (8): 78 % flour sample from bakery C, code (9): 78 % flour sample from bakery D, code (10): 82 % flour sample from bakery E.

Ash Results (Percentage)

The variance analysis of the sample ash results is shown in Table 2, and the comparison of the mean ash results is shown in Table 3 and Figure 2. The samples' variance analysis revealed that the treatment had a significant impact on the amount of ash in the flour samples (P < 0.05). According to the results of comparing the means of the samples, samples 6, 4, 3, and 2 contained the most ash (P < 0.05), and there was no significant statistical difference between
The results of microbial tests of flour samples

The results of the total microbial population (cfu/g) Table 4 presents the variance analysis of the total microbial population of the samples, and Table 7 and Figure 3 present the results of the comparison of the mean of the total microbial population of the samples. The samples' variance analysis revealed that the treatment had a significant impact on total microbial population of the flour samples (P < 0.05). The results of comparing the means of the samples revealed that sample 1 had the highest microbial population, which was significantly higher than that of the other samples (P < 0.05). There was then no statistically significant difference between the mentioned treatments (P > 0.05), and samples 2, 3, and 4 had the highest amounts of microbial population, respectively.

Mold Count Results (cfu/g)

The results of the comparison of the mean of the mold population of the samples are shown in Figure 4, and the variance analysis of the mold population of the samples is shown in Table 5. The samples' variance analysis revealed that the treatment had a significant impact on the mold population in the samples of flour (P < 0.05). The results of comparing the means of the samples revealed that sample 8's mold population was significantly higher than that of the other samples (P < 0.05), and that samples 5, 6, and 10 then had the highest mold populations, with no statistically significant differences between the mentioned treatments being found (P > 0.05).

Table 4: Variance analysis of the total microbial population of flour samples (cfu/g)

<table>
<thead>
<tr>
<th>Sources of Changes</th>
<th>Degree of Freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between treatments</td>
<td>9</td>
<td>14.33</td>
<td>260.09</td>
<td>0.000**</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference at the 5 % probability level

Table 5 Analysis of variance of mold counts of flour samples

<table>
<thead>
<tr>
<th>Sources of Changes</th>
<th>Degree of Freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between treatments</td>
<td>9</td>
<td>0.157</td>
<td>10.094</td>
<td>0.000**</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference at the 5 % probability level

Table 6 Variance analysis of yeast population of flour samples

<table>
<thead>
<tr>
<th>Sources of Changes</th>
<th>Degree of Freedom</th>
<th>Mean square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Treatments</td>
<td>9</td>
<td>0.157</td>
<td>10.094</td>
<td>0.000**</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant difference at the 5 % probability level
Table 7 Results of microbial tests of flour samples

<table>
<thead>
<tr>
<th>Test Sample</th>
<th>Microbial population (cfu/g)</th>
<th>Yeast population (cfu/g)</th>
<th>Mold population (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>code (1)</td>
<td>$7.73 \times 10^2 \pm 9.60$</td>
<td>$7.50 \times 10^2 \pm 7.00$</td>
<td>$0.66 \times 10^2 \pm 1.15$</td>
</tr>
<tr>
<td>code (2)</td>
<td>$4.23 \times 10^2 \pm 4.50$</td>
<td>$6.50 \times 10 \pm 3.00$</td>
<td>$1.00 \times 10^2 \pm 1.00$</td>
</tr>
<tr>
<td>code (3)</td>
<td>$3.66 \times 10^2 \pm 0.09$</td>
<td>$2.50 \times 10 \pm 2.50$</td>
<td>$2.33 \times 10^2 \pm 1.52$</td>
</tr>
<tr>
<td>code (4)</td>
<td>$2.93 \times 10^2 \pm 4.93$</td>
<td>$1.33 \times 10 \pm 4.50$</td>
<td>$2.66 \times 10^2 \pm 0.57$</td>
</tr>
<tr>
<td>code (5)</td>
<td>$1.07 \times 10^2 \pm 0.07$</td>
<td>$1.90 \times 10 \pm 2.64$</td>
<td>$5.66 \times 10^2 \pm 1.52$</td>
</tr>
<tr>
<td>code (6)</td>
<td>$3.00 \times 10^2 \pm 0.00$</td>
<td>$6.50 \times 10 \pm 9.50$</td>
<td>$6.00 \times 10^2 \pm 1.00$</td>
</tr>
<tr>
<td>code (7)</td>
<td>$2.96 \times 10^2 \pm 3.21$</td>
<td>$7.00 \times 10 \pm 4.00$</td>
<td>$1.66 \times 10^2 \pm 1.52$</td>
</tr>
<tr>
<td>code (8)</td>
<td>$2.96 \times 10^2 \pm 3.21$</td>
<td>$7.33 \times 10 \pm 1.15$</td>
<td>$1.00 \times 10^2 \pm 3.00$</td>
</tr>
<tr>
<td>code (9)</td>
<td>$1.90 \times 10^2 \pm 2.00$</td>
<td>$6.66 \times 10 \pm 1.52$</td>
<td>$2.33 \times 10^2 \pm 0.57$</td>
</tr>
<tr>
<td>code (10)</td>
<td>$1.66 \times 10^2 \pm 1.15$</td>
<td>$1.66 \times 10 \pm 1.15$</td>
<td>$7.00 \times 10^2 \pm 1.00$</td>
</tr>
</tbody>
</table>

Different letters indicate significant differences (P ≤ 0.05).

Samples: code (1): 82% flour sample from bakery A, code (2): 82% flour sample from bakery B, code (3): 82% flour sample from bakery C, code (4): 82% flour sample from bakery D, code (5): 82% flour sample from bakery E, code (6): 78% flour sample from bakery A, code (7): 78% flour sample from bakery B, code (8): 78% flour sample from bakery C, code (9): 78% flour sample from bakery D, code (10): 82% flour sample from bakery E.

Yeast Count Results (cfu/g)

The results of the comparison of the mean of the yeast population of the samples are shown in figure 5, and the variance analysis of the yeast population of the samples is shown in table 6 as well. The samples’ variance analysis revealed that the treatment had a significant impact on the yeast population in the flour samples (P < 0.05). The comparison of the sample means revealed that samples 1 and 7 had significantly higher yeast populations than the other samples (P < 0.05), with samples 2 and 6 having the highest yeast populations (P < 0.05). Additionally, there was no statistically significant difference between the mentioned treatments (P > 0.05).

Evaluation of the Results of the Total Microbial Population (Cfu/G)

The average of the samples revealed that each sample had microbial contamination, and sample 1 had the highest microbial population, which was significantly higher than that of the other
samples (P < 0.05). After that, samples 2, 3, and 4 had the highest amount of microbial population, respectively (P < 0.05), and there was no statistically significant difference between the treatments (P > 0.05). The flours with a higher level of extraction appear to have had more microbial contamination. However, according to Iranian national standard No. 2393, the maximum total microbial population of wheat flour is 105 (cfu/g), and all of the samples that were examined fell within the standard range.

**Evaluation of Mold Count Results (Cfu/G)**

The results of comparing the means of the samples revealed that sample 8 had a significantly higher mold population than the other samples (P < 0.05), followed by samples 5, 6, and 10, which had the highest mold populations. However, no statistically significant differences were found between the mentioned treatments (P > 0.05). It appears that higher extraction flours had less mold contamination. On the other hand, the mold population in wheat flour can only be as high as (cfu/g) 103 x 5 in accordance with Iran's national standard No. 2393, and all of the samples that were examined fell within the standard range.

**Evaluation of yeast count results (cfu/g)**

The results of comparing the means of the samples revealed that samples 1 and 7 had significantly higher yeast populations than the other samples (P < 0.05), and there was no significant statistical difference between the mentioned treatments (P > 0.05). The highest yeast populations were found in samples 2 and 6 after that (P < 0.05). The maximum yeast population allowed by Iran's national standard No. 2393 for wheat flour is 103 x 5, and all of the samples that were examined fell within the standard range.

None of the bread samples in the study by Heydari et al. (2017) in Bandar Abbas tested positive for coliform or *Escherichia coli*, but 24% of the bread samples tested positive for mold. In some samples, the investigated flours’ microbial content was above the permitted level (Heidari et al., 2017). According to the study of Nasehi and Tahanejad (2013) on the chemical, sensory, and microbial properties of flour produced in Khuzestan, Setareh and Khabazi flour have acceptable levels of protein, ash, moisture, and pH. Analyzing the microbial properties of various flour types revealed that the average level of mold and yeast contamination in flour, as well as their total count, complied with Iranian national standards. *Escherichia coli* and Salmonella can persist for a very long time in wheat flour stored under typical conditions and used in homes and commercial settings, according to a study by Forghani et al. (2019). *Escherichia coli* contamination in flour can be effectively reduced by heat treatment, but salmonella contamination is more susceptible to this effect. On the other hand, Forghani et al. (2019) found that keeping the flour produced at slightly high temperatures (35 °C) for at least two months prior to distribution can be a successful alternative strategy against Salmonella and *Escherichia coli*. The cases of outbreaks of food-borne diseases and microbial contamination of wheat flour and wheat-based products have been compiled by Harris and Yada (2019). Dough mix contaminated with *E. coli O157:H7* in 2016 and cake mix contaminated with *Salmonella Agbeni* in 2018 are two of the most recent cases related to the consumption of raw wheat flour products in the United States that have been documented (Harris and Yada, 2019). When Myoda et al. (2019) looked at the flour that was sold in the United States, they discovered that Salmonella was present in 1.23 % of the samples and *Escherichia coli* were present in 0.44 % of the samples. While working with flour,
consumers are advised by the US Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention to avoid eating dough and raw dough, wash their hands with soap after handling flour, and keep raw flour separate from prepared foods to avoid cross-contamination (FDA, 2017; Centers for Disease Control and Prevention, 2019).

CONCLUSION

The investigation of microbial contamination of flour from bakeries in Ghaemshahr, Iran was part of the current study. The findings when compared to other similar studies show that the flours distributed in the bakeries of Ghaemshahr city are in good condition in terms of the total microbial population, yeast population, and mold population. Analyzing the microbial properties of various flour types revealed that the average level of mold and yeast contamination in flour, as well as their total count, complied with Iranian national standards.

CONFLICT OF INTEREST

The authors whose names are listed certify that they have no affiliations with or involvement in any organization or entity.

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