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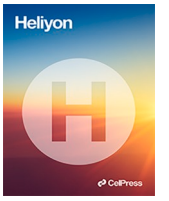

Research article

Investigating the mildew and yeast in the bakeries of Abhar City, Iran

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ABSTRACT

The presence of mold and yeast in bread leads to contamination in bread. Cancer and estrogenic abnormalities in humans are among the diseases caused by fungal and mold mycotoxins in bread.

Bread production waste was estimated at about 30 % in Iran country. A comprehensive and specific image study of the sanitary condition of bread and bakeries in Abhar city has not been done. The purpose of this study was to determine the contamination resulting in yeast and mildew on bread supplied in Abhar City bakeries, to examine the sanitary condition of bakeries, and to provide the food security of Iranians. As well as determining the impact of health status variables and the location of bakeries on mold and fungal contamination. This research was done in the bakeries of Abhar in the period of February 1, 2022 to July 1, 2022. Evaluation of mold and yeast load of 61 breads from 61 bakeries was done using sterile potato dextrose agar with Chloramphenicol plates. In order to check the hygiene status of the bakery environment, modified form of Article 13 with 56 items equal to 56 scores was completed. Variables such as baking soda, sodium dithionite, salt percentage, to evaluate mildew and yeast, and the sanitary condition of bakeries were analyzed. After evaluating health status, the score range was divided based on a 4-point Likert scale. All results were compared with the standard and contamination level of each restaurant. 31 of 60 samples (51.6 %) showed mold and yeast infection. 17 bakeries of the 61 bakeries surveyed (43 %) received 100 % points in the five sections (individual health of 14 items, food hygiene of 10 items, tools and equipment of 16 items, building sanitation of 16 items, and disinfection). 49 samples (80.3 %) of 61 breads evaluated by chemical standard such as amount of baking soda were favorable. Sixty samples (98.4 %) of 61 breads complied with the chemical standard of sodium dithionite. 5 samples (8.3 %) of the 61 breads had excess salt levels. 33 samples out of 61 tested (54.1 %) were infected with mold and yeast. Of these, 26 (78.7 %) belonged to serotypes producing toxin A. The bread quality in some bakeries in Abhar City is poor and infected with mold and yeast. Careful monitoring and monitoring of the health status of bakeries, along with training sessions for bakery staff, will lead to improved baking health.

1. Introduction

Hunger reduction is one of the Millennium Development Goals and food security, as having deep roots in the challenge of poverty,

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is one of the most important strategies to combat hunger [1,2]. Food security plays a key role in maintaining public health [3]. The food consumption pattern program, food production and supply, and the access to adequate, healthy, and desirable food for all members of the society at a reasonable cost is one of Iran's national development plans [1]. Statistics show that household wheat bread waste (HBW), as one of the key items in the food basket, during the grain harvesting process, bread production, and consumption, is about 30 % between 1991 and 2015 in Iran (Equivalent to $ 300 million wasted) [4]. Annual per capita consumption of bread per person is 117 kg in Iran and 25 kg in the world. In Iran, more than 90 % of the energy consumed is supplied from plant foods, the share of cereals is 64 % and the share of bread in urban and rural areas is 40 % and 60 %, respectively. In other words, cereals and bread are the main food of the Iranian people. Rice is the main composition of traditional and ethnic Iranian foods [5]. Lavash Bread has energy, moisture (14.35 %), some types of B vitamins, iron (7.62 μg/g), calcium (27.9 μg/g), and some protein (10–12 %). For example, the amount of calcium in French bread is reported to be 16.54–100.8 mg/100 g [6]. Fungi are the most important contaminating microorganisms and fungi activity leads to decaying bread [7]. The shelf life limit of bread and pasta will be lower (2–5 days) than biscuits and cake (several months) if they attentively pack [8]. Mycotoxins are secondary metabolites and toxic to human and animals produced by filamentous fungi [9]. Penicillium and Roccosum, Aspergillus okraceus, and Aspergillus carbonarius, as a main producer of Ochratoxn A (OTA), and Aspergillus niger produce mycotoxin OTA in cereals and its products such as bread, are known to be the most important source of human contamination, especially in humid conditions exceeding 15-13 % ($C_{20}H_{18}O_6NCl$, as a human carcinogenic, in the Group 2B) [10]. OTA is nephrotoxic mycotoxin with carcinogenic, teratogenic, genotoxic, immunotoxic, mutagenic, and neurotoxic properties [11,12]. The European Union has declared the maximum permitted amount of OTA in cereals and its products at 5 ng/g and index values for triticin toxin [13]. Many researchers have researched mold and yeast in food. Czaban et al. (2015), for example, investigated wheat and found that there was a strong correlation between wheat pollution and the variables of temperature and humidity [14]. Mankevicien et al., in 2014, investigated wheat grown in Lithuania and they announced that wheat was contaminated with triticin, dioxynivalenol, and zearalenone [15]. Therefore, this study was carried out to investigate the bread in Abhar city bakeries in terms of mold and yeast contamination. Variables included health status and location of bakeries on mold and fungal contamination. The theoretical framework is a logical, developed, described, and complete network between the variables provided through processes such as questionnaires, observations, and literature review. Bakery environmental health assessment and determining the load of bread mildew and yeast includes the categories of individual, food stuffs, tools and equipment, and building. The main challenge is the health and sanitary bakery and quality bread, respectively, how are the bakery and bread? Fig. 1 shows the theoretical framework of the research in the form of investigated variables.

2. Materials and methods

2.1. Describe the sampling location

Lack of comprehensive and specific investigation on the sanitary condition of bread and bakeries, access to data, and identification of 9175 citizens suspected of high blood pressure are among the reasons for choosing Abhar city as a research location. Based on the purpose, this research is applied research. Based on the data collection method, this research is an experimental-survey research. Based on the nature of the data, this research is quantitative/semi-quantitative research.

Quantitative/semi-quantitative research is used to accurately determine and estimate the amount of variables. Data can be interpreted with statistical analysis such as analysis of variance. The number of active bakeries, not under repair, and 10 years old, is

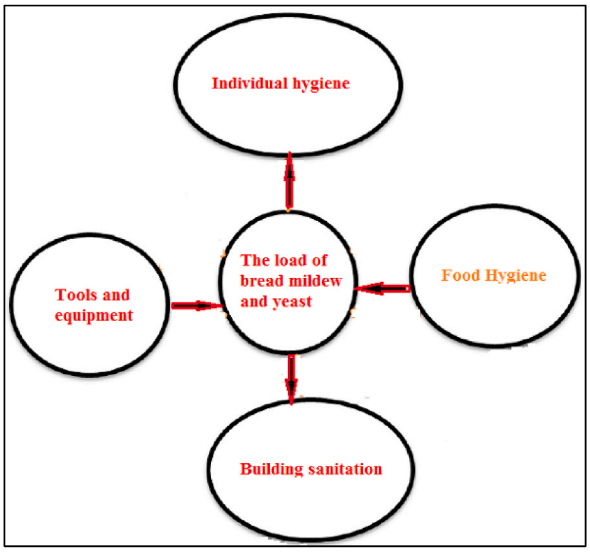

Fig. 1. Conceptual model of factors affecting mildew and yeast load.

72. Based on Cochran's formula and criteria including Z of 1.96, p = q of 0.5, and d of 0.05, the number of samples is 61. In other hands, Based on stratified cluster random sampling, the sampling size of this study is 61 bakeries in Abhar city, as the second most populated city of Zanjan province, in 2022 [16]. The area of Abhar city and its population are about 3362 square kilometers and 151319 people, respectively. Its geographical coordinates range is from 48° 35 min to 49° 25 min east. The geographical coordinates range from 48° 35 min to 49° 25 min east and 35° 50 min to 36° 45 min north of the equator. The height and The average annual rainfall of Abhar city are 1540 m and 300 mm, respectively. The maximum and minimum relative humidity of Abhar city are 94.4 % and 23.3 %, respectively. This area is considered as a semi-cold and dry in Iran country [17].

2.2. Sampling

61 samples of 72 active bakeries, not under repair, and 10 years old (85 %) was selected and with confidence to a great extent represent the whole bakery in Abhar city. In large societies, the results obtained from the sampling method were so accurate that they could be used as the results of the society itself. Because it was possible to train people to prepare questionnaires due to the smaller work load in this research, the accuracy of sampling was more than census. Sampling was done by stratified cluster random sampling in standard conditions and in the interval of February 1, 2022 to July 1, 2022. Sampling was performed between 10 a.m. and 12 a.m. Since part of the research was done during the holy month of Ramadan and the working hours of the bakeries were from 9 to 12:30.

After choosing the bakeries, the bread samples were selected in a proportional class based on the type of baked bread from all selected bakeries (Tafton 1 samples, Lavash 20 samples, Barbari 15 samples, Sengak 23 samples, baguette 2). In order to avoid the interfering factor in the whole research, the sampling time was chosen from 10 to 12. Bread sampling and variable analysis were performed according to the guidelines of the Iranian National Standardization Organization. The specimens were transferred to the laboratory after sampling in sterile polyethylene bag and under appropriate conditions of 4 °C. A sampling integrity is a set of processes that ensure that samples are uniformly sampled, transported and stored. Rules such as sterile conditions, experienced sampler, avoiding the introduction of secondary contamination from the person taking the sample, sampling equipment, and environment to the sample lead to ensuring the absence of human error during sampling, transfer and storage of samples. It was kept frozen at −18 °C until testing. The samples were transferred to the laboratory and analyzed within 2 h after collection [18]. This study does not need the ethical approval for data analysis. The research was done according to the ethical guideline (http://behdasht.gov.ir). Also, informed consent was obtained from the participants. All methods were carried out in accordance with relevant guidelines and regulations.

2.3. Identification of biological agents

First, 1 g of the homogenate sample was added to the test tube containing 9 ml of diluting peptone water and mixed according to modifying serial dilution technique [19]. 1 ml of the diluted material was poured onto a potato dextrose agar plate (PDA, Merck, Germany) with chloramphenicol (Merck, Germany) (dilution plate method) and were incubated at a temperature of 30 ± 2 °C for 4–5 days. PDA was a common medium to grow fungi and presence of nutrient-rich base as increasing spore and pigment production. Also, isolate individual colonies on PDA if further species identification was necessary. Chloramphenicol acted as a selective agent to inhibit the overgrowth of competing microorganisms in mixed samples and also allows selective isolation of fungi. PDA and sabouraud dextrose agar (SDA) culture media were used for mildew and yeast, respectively. 5 g of the of the homogenate sample was added to the 225 ml of peptone water, mixed on an orbital shaker for 20 min at a speed of 100 rpm, added the test tube containing 9 ml of diluting peptone water and mixed according to modifying serial dilution technique [19]. 0.1 ml of the diluted material was poured onto a SDA plate (SDA, Merck, Germany) with chloramphenicol (Merck, Germany) (dilution plate method) and were incubated at a temperature of 25 ± 2 °C for 7 days. Yeast and mildew were counted with Kant colony apparatus (Rocker, Galaxy230 model, Taiwan) [20]. At least three plates including 30–300 colonies were counted due to inferencing strong. Plates with too many or too few colonies should be re-plated from a new dilution. Yeast and mildew were diagnosed after counting by microscope [20]. After incubation, observing creamy white colonies indicated the presence of yeast, and colored filamentous colonies indicated the presence of fungi in the sample. After dying with cotton blue, the morphology including shape, structure of conidia, conidiophores, pigmentation, shape of sporangia, sporangiophores were recorded. The identification was based on the standard keys available.

10 g of frozen samples were homogenized by an electric mill (Molins, France). 5 g of the milled sample was transferred to the test tube and 10 ml of phosphoric acid (Merck, Germany) and 20 ml of dichloromethane (Merck, Germany) were added to it and the tube was centrifuged in a centrifuge (Aika, Germany) with a rotation of 2000×*g* for 5 min. The phosphoric acid supernatant was removed. The substrate suspension was passed through a filter paper (Watman, UK). 12 ml of filtered solution was transferred to the test tube. At 50 °C, it was dried under gentle nitrogen flow. At 50 °C, it was dried under gentle nitrogen flow and the tube was centrifuged in a centrifuge (Aika, Germany) at a rotation of 2000×*g* for 5 min. The hexagonal top layer was removed. 50 μl of substrate suspension was diluted with 200 μl of diluting buffer (Iranian National Standardization Organization). The amount of adsorbed OTA in the prepared sample was measured by ELISA (England State Fax2000) according to the kit manufacturer's instructions (Kit Reidskreen, Biofarm Co., Germany) at a wavelength of 450 nm. The specifications of kit were limit of detection (LOD) of 1.7 μg/kg, sensitivity of 0.4 ppb, coefficient variation of<10 %. The accuracy and precision of the selective test method, equipment and materials, the performance of laboratory personals, application of quality control strain prepared from reputable centers such as *Candida albicans* were the purpose of the quality control program during examining analysis. Also, as a general rule, for a series of 100 or less, 3–5% of the tubes and plates should be checked for contamination and bacterial growth. The results of mold and yeast count in CFU/g and the results of OTA toxin concentration in ng/g were reported [21]. The salt of the baked bread sample was measured according to the National Standard Organization of Iran (No. 2628). pH < 6.3 is a sign of not using baking soda in bread and salt more than 2.3 % is a sign of using salt in

large quantities. The sodium dithionite of the baked bread sample was measured according to the National Standard Organization of Iran (No. 2628) by spectrophotometer at wavelength of 560 nm. According to the standard the bread should not have blanket. The specifications of this method was LOD of 1 mg/l, sensitivity of 0.4 ppb, coefficient variation of<10 %. The method fitted a linear model with $R^2 = 0.98$. 10 g of the homogenate sample was added to the 100 ml balloon, 4 ml of sodium hydroxide solution (0.1 M) was added, added 96 ml deionized water. The blank sample was prepared with flour and dough without sodium dithionite. After centrifuging both samples, 2 ml of rosanilin solution and 2 ml of formaldehyde were added to the test and sample tubes containing 10 ml of filtered solution, and after 30 min, the absorption value was read at 560 nm by the spectrophotometer.

2.4. Bakery health assessment

The collection tool is a modified form of bakery environmental health assessment (https://markazsalamat.behdasht.gov.ir), which were completed in 61 bakeries within 40 min. Application of a modified form of bakery environmental health assessment led to improving of bakery sanitation and hygiene indexes and indicating existing measures for consumer health. For example, installation of the first aid box in a suitable and accessible place (section individual hygiene), the pH value of the bread should be 6.2 (section food hygiene), the kneader equipped with wire mesh (section tools and equipment sanitation), and water temperature of 22–27 °C (section building sanitation). Each item of environmental health assessment form was adjusted based on a two-part scale (conformity and non-conformity with item). And in order to calculate the score based on the said scale, score 1 was assigned to conformity. The maximum score assigned to the correct answer to the evaluation form is 56. The Likert scale was divided into excellent (42–56), good (28–41), moderate (14–27) and weak (0–13) scales based on the Likert scale. The categories for the bakery environmental health assessment form included individual hygiene (14 items), tools and equipment sanitation (16 items), food hygiene (10 items), and building sanitation (16 items). Collected data were analyzed by SPSS 18 software. The statistical analysis was done based on quantitative variables after transforming all of quantitative and qualitative variables into quantitative. To validate the form, content validity was used by ten restaurant owners and content validity index was calculated (23). Its validity was obtained and confirmed using content validity index (CVI = 0.82). Calculating CVI required asking experts to determine the relevance degree of each item based on a four-part spectrum of unrelated, needing basic revision, relevant but needing revision, and completely relevant. The number of experts who chosen options 3 and 4 by the total number of experts. A value < 0.7 was rejected, a value of 0.7–0.79 should be reviewed, and a value > 0.79 was acceptable. To reliable the questionnaire, test-retest method reliability was used by 4 owners in the pilot test. Its reliability was obtained and confirmed using Kuder-Richardson (r = 0.80) [22]. The Kuder-Richardson Coefficient was a method used to assess the internal consistency of a measure, particularly with dichotomous data. It was based on the variance of the item scores and was the dichotomous equivalent to the coefficient alpha. Calculating the reliability of the questionnaire with Kuder-Richardson method required having the variance of the total and average scores. When choosing the bakeries, the confounding factors including to active, not be under repair, and 10 years old of bakery were considered. ANOVA test at a significant level of 0.05 was used and was chosen for statistical analysis of variance (P value < 0.05) (SPSS version 18) due to being the normal distribution of data and the absence of outliers [23]. ANOVA test was used to compare the average amount of mildew and yeast with other research parameters. Frequency, percentage, minimum, maximum, mean, and standard deviation were used to describe the data. Map preparation of restaurant location in Abhar city was determined by Arc GIS 10.1 [24].

3. Results

3.1. Checklist evaluation of health inspection

According to the results of the Bakery Health Status Assessment Form, the average environmental health score of 61 bakeries is as follows: in the individual health category, 10.1 ± 2.4 (good, equal to 72 %), in the tools and equipment item, 12.7 ± 1.6 (excellent, equal to 79 %), in the food item 6.0 ± 1.1 (mean, equal to 60 %), and in the construction item, 12.4 ± 1.9 (excellent, equal to 76 %) (Tables 1 and 2).

3.2. Microbial and chemical evaluation of food

49 samples (80.3 %) of 61 breads evaluated by chemical standard such as amount of baking soda were favorable. Sixty samples (98.4 %) of 61 breads complied with the chemical standard of sodium dithionite ($Na_2S_2O_4$). 5 samples (8.3 %) of the 61 breads studied had excess salt levels (Table 3). 33 samples out of 61 tested (including 23 Sangak, 20 Lavash, 15 Barbari, 2 Baguette and 1 Taftoon) (54.1 %) were infected with mold and yeast. Of these, 26 (78.7 %) belonged to serotypes producing toxin A (Table 4). Statistical

Table 1
Frequency of health status of bakeries in Abhar city.

Health score range (number, %)					
No.	Weak (0–10)	Moderate (11–22)	Good (23–31)	Excellent (32–42)	Total (Number, %)
1	0 (0/0)	0 (0/0)	33 (54.1)	28 (45.9)	61 (100)
Total	0 (0/0)	0 (0/0)	33 (54.1)	28 (45.9)	61 (100)

Table 2
Frequency of compliance with health status items of bakeries in Abhar City.

Health category (Number of items)	Weak (0–10)	Moderate (11–22)	Good (23–31)	Excellent (32–42)	Total (Number, %)	Mean	Limitation (Minimum-Maximum)
Individual (14)	0 (0.0)	6 (9.8)	24 (39.3)	31 (50.9)	61 (37.0)	10.1 ± 2.4	(5–14)
Food stuffs (10)	0 (0.0)	6 (9.8)	49 (80.4)	6 (9.8)	61 (64.0)	6.0 ± 1.1	(4–10)
Tools and equipment (16)	0 (0.0)	1 (1.6)	11 (18.0)	49 (80.4)	61 (74.0)	12.7 ± 1.6	(6–16)
Building (16)	0 (0.0)	1 (1.6)	15 (24.6)	45 (73.8)	61 (95.0)	12.4 ± 1.9	(7–16)
Disinfection	0 (0.0)	6 (9.8)	24 (39.3)	31 (50.9)	61 (37.0)	10.1 ± 2.4	(5–14)

comparison of mildew and yeast infection variables and the variables studied, such as the location of the bakery, are shown in Table 5. As shown in Table 5, there was a significant correlation between the number of yeast and mildew with the health status, sodium dithionite, and location of the bakeries, ($P < 0.05$) (Table 5). The minimum levels of different sources of mold and yeast in the bakery are shown in Table 6. As seen in the table, 4 sources of mold and yeast were identified in Abhar bakery. Their range in percentages included food hygiene (35.5–36.9), personal hygiene (33.33–7.9), building sanitation (24.24–3.5) and tools and equipment sanitation (4.5, 4.7). Fig. 2 shows the distribution map of health status of bakeries in Abhar city.

4. Discussion

According to the results of the environmental health survey of 61 bakeries, it can be concluded that food hygiene is less in line with Article 13 of the Food, Beverage, Cosmetics and Health Law. Therefore, measures should be taken to improve it by holding courses for bakeries staff. In other words, food hygiene training can improve the health of the bakery. Daily intake main foods including fruits and vegetables confirms a strong diet with an equilibrium mineral and vitamin intake [25]. Goodarzi et al. (2014) Showed that 65.7 % of the bakeries studied in Bandar Abbas city were poor in environmental health status [26]. Sadeghizadeh-Yazdi et al. (2020) reported that the improvement of environmental conditions, especially in bakeries in Yazd city, as one of the most sensitive places, was significant after the training [27]. The health status items of fantasy bakeries in this research is better than traditional bakeries. In other hands, ignoring hygiene principles, especially in traditional bakeries leads to the creation of unhealthy disorders in consumers and the unhygienic supply of bread, which is the dominant strength of the people. Fantasy bakeries are more effective in increasing food security than traditional bakeries. Ignoring cleanliness and personal hygiene by bakery personnel leads to a decrease in customers. Padmavathi and Praveen (2023) reported the same result [28]. In the individual health category in this research is good due to compliance with the principles of personal hygiene resulting from previous training by environmental health inspectors. Amini-Rarani et al. (2021) reported that the social health of bakeries in Isfahan city was low and middle [29]. In the individual health category including wearing uniform and hat in this research is good. In other words, all the workers of the surveyed bakeries are dressed in uniforms and hats. Abd El Monem et al. (2022) reported that 48.9 % of surveyed bakers in Port Said city were not dressed in uniforms [30]. Emmanuel and Sussan (2014) reported that most of the participants were frequently dressed in uniform such as apron [31]. In the individual health category including having health card in this research, all bakery personnel have a health card (both valid and invalid). Lawel et al. (2022) reported that 12.5 % of surveyed bakers in Tamale metropolis, Gana did not have a health certificate [32]. Measurement errors can be minimized by careful control and the use of experimental design methods.

Absolute error and relative error for critical cases were obtained as 7.2 and −0.8 respectively. Absolute error and relative error for non-critical cases were obtained as 25.7 and −0.74 respectively. Potential sources of error include random and systematic. Sampling error is one of the types of random errors that the sample size has good statistical power (alpha = 0.05). Systematic error includes selection bias (not present due to inclusion criteria), measurement bias (probably absent due to choosing the stratified cluster random sampling), and observer bias (not present due to the double-blindness of the study).

The amount of baking soda, sugar inks, and salt percentage in the examined bread samples is desirable in 80.3, 98.4, and 8.4 % cases, respectively. Abolli et al. (2021) reported that amount of salt in 16.3 % of surveyed breads in Garmsar county were desirable [33]. Loloei et al. (2019) reported that amount of salt, as a flavor enhancer, in the types of breads surveyed were inappropriate in different regimes of Iran country and it can be considered as one of the effective factors in increasing the prevalence of non-communicable diseases such as high blood pressure [34]. The baker must have the necessary knowledge and skill for the amount

Table 3
Frequency of compliance with bread standard in Abhar city.

Health limitation	Desirable	Undesirable	Suspicious	Total (number, %)
Baking soda	49 (80.3)	9 (14.8)	3 (4.9)	61 (100)
pH	49 (80.3)	9 (14.8)	3 (4.9)	61 (100)
Sodium dithionite	60 (98.4)	1 (1.6)	0 (0.0)	61 (100)
Salt percentage	5 (8.2)	56 (91.8)	0 (0.0)	61 (100)

Table 4
Frequency of compliance with mold and yeast consumption bread in Abhar City.

No.	Bread type	Mold and yeast limitation (1000 CFU/g)			Mean number (CFU/g)	Mean value of Ochratoxin toxin (ng/g)
		Allowed	More than allowed	Total (number, %)		
1	Barbari	9 (14.8)	6 (9.8)	15 (24.6)	158.2 (7–620)	1.5 ± 2.4 (0.07–6.2)
2	Sangak	14 (23.0)	9 (14.7)	23 (33.7)	160.6 (10–75)	1.6 ± 2.1 (0.1–7.5)
3	Lavash	4 (6.6)	16 (26.2)	20 (32.8)	237.0 (15–800)	2.3 ± 2.6 (0.15–8.0)
4	Fantasy	1 (1.6)	1 (1.6)	2 (3.2)	255.0 (20–490)	2.5 ± 2.9 (0.2–4.9)
5	Taftoon	0 (0.0)	1 (1.7)	1 (1.7)	390.0	3.9
	Total	28 (46.0)	33 (54.0)	61 (100)	191.9 ± 4.4	2.3 ± 1.9

Table 5
Comparison of mold and yeast score variability and bread contamination information in Abhar.

No.	Variable	R^2	ANOVA (P-Value)
1	Sodium dithionite	0.991	0.001 (S.)
2	pH	0.134	0.305 (N.S.)
3	Backing soda	0.149	0.252 (N.S.)
4	Salt percentage	0.176	0.176 (N.S.)
5	Breed type	0.182	0.16 (N.S.)
6	Backery position	0.616	0.001 (S.)
7	Health score	0.809	0.001 (S.)

Table 6
The emission sources of mold and yeast in Abhar city bread.

No.	Source name	Mean (%)	Limitation
1	Health Food	37.2	(35.5–36.9)
2	Personal hygiene	33.8	(33.7–33.9)
3	Building sanitation	24.4	(24.3–24.5)
4	Tools and equipment sanitation	4.6	(4.5–4.7)

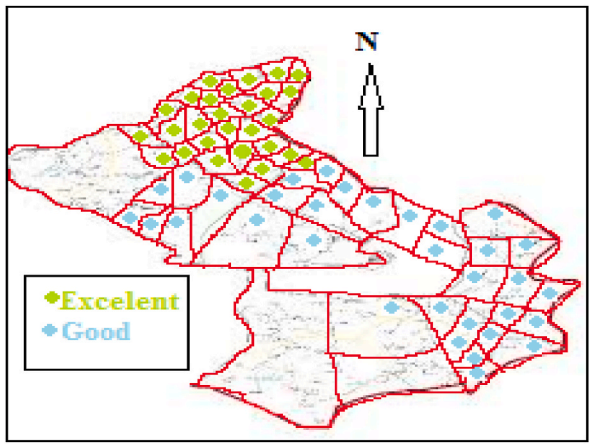

Fig. 2. Distribution map of health status of bakeries in Abhar city.

of salt added to the bread according to the type of bread, which in turn is effective in promoting public health. It is concluded that the high amount of salt in bread can lead to increasing the prevalence of blood hypertension due to its relationship with salt intake. This research finding is consistent with the research conducted in Lima, Peru by Saavedra Garcia et al. (2016). They were found that there was confirmed relationship salt intake amount with occurrence illnesses such as cardiovascular and kidney [35]. According to world health organization (WHO) recommends, daily intake of sodium/salt in adult is 2 g (5 g) due to decreasing prevalence of diseases [36]. Therefore, it is necessary to inform and train the public about the potential threats of additives such as baking soda and salt. In other words, food hygiene training, such as paying attention to the date of flour consumption, consuming better quality flour and not packing hot bread in inappropriate packaging can lead to improved food hygiene. Garcia and Copetti (2019) showed that temperature and relative humidity of the environment and product water activity (aw) were crucial and main variables for fungal growth in bakery

products [37]. Due to the excessive number of molds and yeast in 61 samples examined, improving the temperature and humidity conditions of the flour, transportation, disinfection of storage space, lack of proximity to storage, rapid flour consumption, proper brand selection of flour and proper packaging are essential. Bryla et al. (2016) reported that toxins produced by Fusarium were the main pollutants in 147 samples of maize examined in Poland [38]. Demissie and Natea (2018) reported than the mean molds count in 90 breed samples in Jimma town were 4.0 ± 0.6 CFU/g [39]. Minutillo et al. (2022) reported that wholemeal flours in Calabria yielded the most number of CFU (527–1840 CFU/g) [40]. Bol et al. (2016) showed that the amount of OTA was reduced up to 6 % due to fermenting in bread samples in Porto Alegre, Brazil [41]. Thirty-three samples out of 61 tested, including 6 barbari, 9 sangak, 16 lavash (maximum), 1 baguette (minimum), and 1 Taftoon (54 %) had over-mold and yeast. Therefore, it can be concluded that an increase in the number of molds and yeast leads to a decrease in the shelf life of lavash bread. Pre-tests showed that increasing the shelf life of the bread led to an increase in the number of molds and yeast due to increased moisture and water activity in the bread. The increase in the number of molds and yeast and the concentration of OTA toxin can be attributed to the higher moisture and water activity of lavash bread. Diagnostic tests showed that Penicillium and Roccosum and Aspergillus okraceus are among the major breadwinner fungi and producers of toxin OTA from the view point of cold weather conditions in Abhar city. Zachariasova et al. (2014) showed that different genera of Fusarium are the most important fungal contaminant and toxin generator in temperate climates [42]. The pre-test of indoor air sampling of bakeries showed the average number of penicillium and rocosom and Aspergillus okraceus species at CFU/m^3 of 845 and 635, respectively. It can be concluded that the optimum temperature of 20 (15–30 °C) along with high long-term moisture inside the bakery leads to the expansion of toxin accumulation in bread. El Chami et al. (2022) showed that temperatures of 15–20 °C as a climatic factor in determining fungal occurrence along with high long-term moisture, as an indicator of fusaris, led to increasing toxin accumulation in wheat over the flowering or clustering period of wheat and reducing wheat quality [43]. In other words, it can be concluded that 33 out of the 61 samples tested had overdose (5 ng/g) of OTA (0.07–0.08 ng g). It can be concluded that the presence of OTA, a major threat to food security, leads to food poisoning among bakery customers. Therefore, it can be concluded that observing the following may reduce the risk of food poisoning caused by OTA: control of hygiene, observance of quality principles during storage, proper storage of flour until consumed, and supervision of farming operations such as the use of equipment and detergents to prevent fungus growth and shelf-life due to the increased relative humidity of the bakery environment. Controlling air flow rate and air pressure inside the bakery results in the prevention of airborne pathogens entering the bakery. It can be concluded that the low velocity of airflow inside the bakery leads to the suction and entry of airborne pathogens from outside into the bakery due to the lack of positive pressure. Colovic et al. (2019) reported that thermal process, additives, stabilizing agents, and fermentation were co-factors in the reduction OTA by temperature [44]. It can be concluded that Taftoon contamination with mold and yeast and OTA are 2.4 and 2.5 times higher than barbari, respectively. Lower mildew and yeast and lower concentrations of OTA in barbari can be attributed to variables such as baking temperature, bread storage at optimum temperature and moisture conditions. It can be concluded that barbari breads are high risk foods. Higher mildew and yeast and higher concentrations of OTA in sangak breads can be attributed to less binding of OTA with lactic acid bacteria (LAB), as bio-preservation agents. This research finding is compatible with the research done by Chiiotta et al. (2016) in Latin America [45] and Bartkiene et al. (2019) in Lithuania [46]. Statistical comparison showed that there was a statistically significant difference between the number of molds and yeast and the variables of health status, location, and sugar inks content ($P < 0.05$). Therefore, it can be concluded that there is a positive correlation between the number of mildew and yeast and the variables of health status, location and essence of sugar. Esfandiari et al. (2013) showed that there was a relationship between the number of microbes in food, staff cleaning, and effective washing and disinfection of surfaces [47]. Pre-test of temperature and moisture content of bakeries showed a positive correlation between number of molds and yeasts and variables of temperature and geographical location of health status, location and sugar inks of bread. The highest number of bread and yeast contaminants and OTA were found in bakeries located south of Abhar. It can be concluded that the trend of spatial fluctuations in the number of microbes in the breads depends on the lack of personal hygiene, nutrition, equipment and buildings. Pre-tests showed that observance of personal hygiene standards, food, equipment washing, attention to physical indoor air variables such as temperature, humidity, ventilation and speed, and suitable flour storage conditions in bakeries located south of Abhar city are of poor quality. Failure to comply with personal hygiene standards, food, equipment and buildings results in secondary food contamination. Pinotti et al. (2016) showed that there is a trend of spatial variations due to seasonal and local climatic conditions in the investigated nutrient types (OTA concentration) around the world [48]. Based on the results of the standard microbial food survey, 1000 molds and yeasts per gram of bread and 5 ng per gram of crude OTA are allowed. Woo and El-Nezami (2016) showed that the daily intake of OTA in pregnant women was 3.38 ng/kg bw/day in Egypt [49]. Based on the results of the location survey of the bakeries, it can be concluded that increasing bread consumption in the south of Abhar city due to more bread purchased by the customer leads to an increase in the number of mold and yeast in the bread. Pre-tests showed that by failing to comply with the HACCP Seven Principles, inadequate washing of containers and surfaces, the number of mold and yeast cultivation tests for washed dishes and fusarium-positive levels were higher in bakeries located south of Abhar than in other areas. It can be concluded that monitoring, regular inspection and proper implementation of the HACCP Seven Principles in the training of bakery staff leads to the improvement of bakery health. Jubayer et al. (2022) found that the proper implementation of the HACCP seven principles, in cake manufacturing company in Dhaka, Bangladesh, led to preventing produce substandard products, detecting source of contamination and saving resources including material, manpower, and finance and material [50]. Kamboj et al. (2020) found that the correct implementation of the HACCP seven principles was why to increase and maintain food safety and hygiene [51]. Muhammad et al. (2023) reported that bread samples from the bakeries were contaminated with specially Penicillium spp., Aspergillus spp., Rhizopus spp., and Fusarium spp [52]. Pre-tests showed that flour distributed (Mahdasht, Sefid Zanjan and Nakhir brands) in Abhar city comply with all physicochemical and microbial standards. Identification of mold and yeast pollutant emission sources are among the strengths of the research. Implementation HACCP system of bread production is suggested as follow:

1 Conduct a hazard analysis include biological (such as mold and yeast), chemical (such as salt), and physical (suitable cooking temperature).
2 Identify critical control points includes bread baking temperature, salt percentage, baking soda, sodium dithionite, and pH; disinfection of hands, surfaces, tools, and use of disposable gloves; type of equipment; water supply system; Sink equipped with hot and cold water faucet; the health of the refrigerator and freezer; observing the cold chain; separation of the food storage area from the detergent storage area; no use of preservatives.
3 Establish critical limits includes compliance with 70 % of non-critical cases and 100 % of critical cases; pH < 6.2; salt 1 %; no sodium dithionite.
4 Monitor critical control points is done with the help of physical and chemical methods.
5 Establish Corrective actions.
6 Establish Verification procedure includes random sampling and removal of defective bread.
7 Establish record keeping procedure.

Examining Iran's policy on health crimes is a sign of the influence of pragmatism in the field of health crimes for various reasons, including media coverage of health crimes, politicization of reactions, and weak public awareness. Therefore, it is necessary to investigate the consequences of applying pragmatic policy in health crimes in different fields of environmental health conditions of bakeries and bread. Due to the contamination of some bread samples under investigation, it is recommended that public health decision-makers apply stricter rules and monitoring in line with personal hygiene and improving the sanitary condition of the bakery and improving the sanitary condition of the bakery and employees can be trained in food safety.

One of the limitations of this research is the high cost of microbial sampling. Failure to investigate mineral (such as heavy metal), organic (such as polycyclic aromatic hydrocarbons, PAHs, PAH4), and other microbial pollutants are among the limitations of this study.

5. Conclusion

- Total health status of bakeries is good (54.1) and excellent (45.9) in Abhar city.
- The score of compliance with health status items of bakeries including individual, food stuffs, tools and equipment, building, and disinfection is 10.1 ± 2.4, 6.0 ± 1.1, 12.7 ± 1.6, 12.4 ± 1.9, and 10.1 ± 2.4, respectively.
- The compliance with bread standards including baking soda, sodium dithionite, and salt percentage is desirable in 80.3, 98.4, and 8.2 % of cases, respectively.
- Compliance with mold and yeast consumption bread is allowed in 46 % of cases. Mean number of mold and yeast and OTA in breads is 1919.3 ± 4.4 CFU/g and 2.3 ± 1.9, respectively.
- According to the results, the number of molds and yeast and toxin OTA were exceeded in 54 % and 19 % of the breads, respectively. Therefore, consumption of contaminated bread can threaten the consumer's health in the long term.
- Statistical comparison showed that there was a statistically significant difference between the number of molds and yeast and the variables of health status, location, and sugar inks content ($P < 0.05$).
- Health food is the most important emission source of mold and yeast in bread.
- In view of spatial distribution of health status in bakeries, the number of mildew and yeast and the concentration of OTA increased with increasing bread consumption.
- Considering the high consumption of bread in the country, people's preference for traditional breads, and the prevalence of infectious and non-infectious on a global scale, it is necessary to pay more attention to the discussion of environmental health, from the production chain to the consumption of food.

Managing cooking, selecting healthy ingredients, regulating the amount of salt and backing soda used, and improving health and sanitation indexes are effective measures in reducing mildew and yeast concentrations in bread. This approach contributes to producing a safer and healthier bread product for consumers. It is noteworthy that all the influencing factors on the amount of mildew and yeast in bread have not been investigated. Therefore, other factors that can possibly affect the amount of mildew and yeast, such as the load of hand microbial, are suggested for future research. It is recommended for future studies to study residues of other contaminants in bread. In view of the food basket and the growing risk index, it is recommended for future studies to carry out detailed research on the quality of flour, soil, and irrigation water; to determine the relationship between the concentration of ochratoxin in bread and the health of people; and the possibility of contracting cancer by different sensitive age groups due to the consumption of contaminated bread.

This research has no direct benefit for the participants but their participation in research can help improve the health status of bakeries. For example, increasing public health literacy about food safety through media; holding educational workshops under the supervision and corporation of the Environmental Health Unit, as a regulatory agency, about sanitation and hygiene in bakery. The result of this study will be printed on the journal in twenty-four months.

Ethical statement

The study complies with all regulations and confirmation that informed written consent was obtained from the participants in collecting data.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Giti Kashi: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation.

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.heliyon.2024.e37956.

References

[1] L. Barosh, et al., The cost of a healthy and sustainable diet–who can afford it? Aust. N. Z. J. Publ. Health 38 (1) (2014) 7–12.
[2] J.L. Sanchez Garcia, I. Beiro Perez, J.M. Diez Sanz, Hunger and sustainability, Economic research-Ekonomska istraživanja 32 (1) (2019) 850–875.
[3] M.B. Ghalibaf, M. Gholami, N. Mohammadian, Stability of food security in Iran; challenges and ways forward: a narrative review, Iran. J. Public Health 51 (12) (2022) 2654.
[4] S. Ghaziani, et al., Household food waste quantification and cross-examining the official figures: a study on household wheat bread waste in Shiraz, Iran, Foods 11 (9) (2022) 1188.
[5] V.M. Karizaki, Ethnic and traditional Iranian rice-based foods, Journal of Ethnic Foods 3 (2) (2016) 124–134.
[6] T.A.S. Gusmão, et al., Mineral content in French type bread with sodium replacement using fluorescence spectrometry X-rays by energy dispersive, Afr. J. Biotechnol. 14 (51) (2015) 3333–3339.
[7] F. Illueca, et al., Bread biopreservation through the addition of lactic acid bacteria in sourdough, Foods 12 (4) (2023) 864.
[8] I. Saeed, et al., Assessment of mold and yeast in some bakery products of Lahore, Pakistan based on LM and SEM, Microsc. Res. Tech. 82 (2) (2019) 85–91.
[9] W.K. Balwan, N. Saba, S. Kour, Study of impact of mycotoxins on the human health, Sch Bull 9 (2) (2023) 19–23.
[10] P. Mozaffary, J.M. Milani, A. Heshmati, The influence of yeast level and fermentation temperature on Ochratoxin A decrement during bread making, Food Sci. Nutr. 7 (6) (2019) 2144–2150.
[11] A. Heshmati, et al., Co-occurrence of aflatoxins and ochratoxin A in dried fruits in Iran: dietary exposure risk assessment, Food Chem. Toxicol. 106 (2017) 202–208.
[12] T. Kőszegi, M. Poór, Ochratoxin A: molecular interactions, mechanisms of toxicity and prevention at the molecular level, Toxins 8 (4) (2016) 111.
[13] L. Wang, et al., Bio-control on the contamination of Ochratoxin A in food: current research and future prospects, Curr. Res. Food Sci. 5 (2022) 1539–1549.
[14] J. Czaban, et al., Colonisation of winter wheat grain by Fusarium spp. and mycotoxin content as dependent on a wheat variety, crop rotation, a crop management system and weather conditions, Food Addit. Contam. 32 (6) (2015) 874–910.
[15] A. Mankevičienė, D. Jablonskytė-Raščė, S. Maikštėnienė, Occurrence of mycotoxins in spelt and common wheat grain and their products, Food Addit. Contam. 31 (1) (2014) 132–138.
[16] K. Jafari, et al., Groundwater quality assessment for drinking and agriculture purposes in Abhar city, Iran, Data Brief 19 (2018) 1033–1039.
[17] M. Yousefi, et al., Data on trend changes of drinking groundwater resources quality: a case study in Abhar, Data Brief 17 (2018) 424–430.
[18] P. Qajarbeygi, M. Kazeminia, R. Mahmoudi, Determine the quality of bread samples used in Qazvin, Iran, J. Chem. Health Risk 8 (1) (2018) 1–8.
[19] J. Ajith, M. Sunita, A study on bread mould spoilage by using lactic acid bacteria and yeast with antifungal properties, Journal of Nutritional Ecology and Food Research 4 (2) (2017) 128–130.
[20] N. Ravimannan, P. Sevvel, S. Saarutharshan, Study on fungi associated with spoilage of bread, Int. J. Adv. Res. Biol. Sci 3 (4) (2016) 165–167.
[21] K. Gholamreza, M.S. Ali, N. Hasan, Determination of aflatoxin in pistachio samples by ELISA technique in Torbat-e-Heydarieh (North-east of Iran), Bull. Env. Pharmacol. Life Sci 4 (2015) 20–22.
[22] G. Kashi, N. Nourieh, Investigation of paramedics, attitude, knowledge and practice regarding the Middle East respiratory syndrome coronavirus (MERS-CoV) in Iran, Journal of Health Sciences & Surveillance System 11 (2023) 147–155, 1 (Supplement)).
[23] G. Kashi, K.K. Doost, Comparison of the effect of lecture and video projector teaching methods on students' attitude, knowledge and practice, International Research Journal of Teacher Education 2 (3) (2015) 30–35.
[24] G. Kashi, F. Khoshab, An investigation of the chemical quality of groundwater sources, Donnish Jour. Res. Environ. Stud 2 (3) (2015) 18–27.
[25] L. Aljerf, N. Aljerf, Food products quality and nutrition in relation to public. Balancing health and disease, Prog. Nutr. 25 (1) (2023).
[26] A. Madani, et al., Hygiene status in urban bakeries of Bandar Abbas in 2012, Journal of Preventive Medicine 1 (1) (2014) 10–15.
[27] J. Sadeghizadeh-Yazdi, Z. Rahaei, The effect of training courses on awareness, attitude, and performance of confectionery, bakery, and restaurant workers in Yazd city, J. Environ. Health Sustain. Dev. 5 (2020) 1021–1029.

[28] D. Padmavathi, D. Praveen, Food sanitation and hygiene in bakery- from farm to fork. International International Journal of Progressive Research in Engineering Management and Science 3 (1) (2023) 148–155.
[29] M. Amini-Rarani, S.H. Abutoraabi, M. Nosratabadi, The role of social health and demographic factors in bread quality: an ecological study in Isfahan, Iran, J. Food Qual. 2021 (2021) 1–6.
[30] H. Abd El Monem, et al., Occupational health hazards and safety measures among bakery workers at Port Said City, Port Said Scientific Journal of Nursing 9 (1) (2022) 330–352.
[31] N. Aguwa Emmanuel, U. Arinze-Onyia Sussan, Assessment of baking industries in a developing country: the common hazards, health challenges, control measures and association to asthma, Age 15 (2014) 21–25.
[32] M. Lawal, et al., The sanitary conditions of bakeries and food safety practices assessment of bakers in Tamale metropolis, Ghana, European Journal of Nutrition & Food Safety 14 (1) (2022) 13–19.
[33] S. Abolli, J. Kazemi, Z.H. Motlagh, Evaluating the quality of bread; based on the amount of salt and baking soda consumption, J. Community Health Res. (2021) 33–40.
[34] S. Loloei, et al., Policy analysis of salt reduction in bread in Iran, AIMS Public Health 6 (4) (2019) 534–545.
[35] L. Saavedra-Garcia, et al., Reducing salt in bread: a quasi-experimental feasibility study in a bakery in Lima, Peru, Publ. Health Nutr. 19 (6) (2016) 976–982.
[36] T. Partearroyo, et al., Sodium intake from foods exceeds recommended limits in the Spanish population: the ANIBES study, Nutrients 11 (10) (2019) 2451.
[37] M.V. Garcia, M. Copetti, Alternative Methods for Mould Spoilage Control in Bread and Bakery Products, Int. Food Res. J. 26 (2019) 737–749.
[38] M. Bryła, et al., Occurrence of 26 mycotoxins in the grain of cereals cultivated in Poland, Toxins 8 (6) (2016) 160.
[39] S. Demissie, G. Natea, D. Raga, Microbial quality and safety of bread sold in cafeteria, tea and bread shop of Jimma town, Oromia regional state, southwest Ethiopia, Int. J. Adv. Res. 6 (1) (2018) 771–779.
[40] S.A. Minutillo, et al., The Fungal microbiome of wheat flour includes potential mycotoxin producers, Foods 11 (5) (2022) 676.
[41] E.K. Bol, et al., Estimated exposure to zearalenone, ochratoxin A and aflatoxin B1 through the consume of bakery products and pasta considering effects of food processing, Food Chem. Toxicol. 89 (2016) 85–91.
[42] M. Zachariasova, et al., Occurrence of multiple mycotoxins in European feedingstuffs, assessment of dietary intake by farm animals, Anim. Feed Sci. Technol. 193 (2014) 124–140.
[43] E. El Chami, et al., Influence of growing season, nitrogen fertilisation and wheat variety on Fusarium infection and mycotoxin production in wheat kernel, Acta Aliment. 51 (2022) 282–289.
[44] R. Čolović, et al., Decontamination of mycotoxin-contaminated feedstuffs and compound feed, Toxins 11 (11) (2019) 617.
[45] M.L. Chiotta, et al., Aspergillus and ochratoxin A in Latin America, Microbes in Food and Health (2016) 265–287.
[46] E. Bartkiene, et al., Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation, Microorganisms 8 (1) (2019) 64.
[47] Z. Esfandiari, et al., Simultaneous determination of sodium benzoate, potassium sorbate and natamycin content in Iranian yoghurt drink (Doogh) and the associated risk of their intake through Doogh consumption, Iran. J. Public Health 42 (8) (2013) 915.
[48] L. Pinotti, et al., Mycotoxin contamination in the EU feed supply chain: a focus on cereal byproducts, Toxins 8 (2) (2016) 45.
[49] C.S.J. Woo, H. El-Nezami, Maternal-fetal cancer risk assessment of Ochratoxin A during pregnancy, Toxins 8 (4) (2016) 87.
[50] M.F. Jubayer, et al., Implementation of HACCP management system in a cake manufacturing company in Dhaka, Bangladesh: a case study, J. Food Qual. 2022 (2022) 321333.
[51] S. Kamboj, et al., Food safety and hygiene: a review, Int. J. Chem. Stud. 8 (2) (2020) 358–368.
[52] S. Muhammad, S. Galadima, Determination of microbiological quality of bread and sanitation conditions of local bakeries in Aliero town, Kebbi state, Applied Science and Technology Research Journal 1 (2) (2022) 1–9.