Microbial and Fungal Contamination of Wheat Flour, Dough, and Bread Samples Collected from Isfahan, Iran

Roya Abedi¹, Masoud Sami¹*, Rasoul Mohammadi² and Maryam Mirlohi¹

¹Department of Food science and Technology, School of Nutrition and Food Science, Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
²Department of Medical Parasitology and Mycology, Infection Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

The present investigation was done to evaluate the microbial and fungal contamination and also molecular identification of fungal species in flour, dough and bread samples collected from Isfahan, Iran. Two-hundred and forty different types of cereals including flour, dough and bread were collected. Samples were subjected to microbial and fungal counting and also PCR-based identification of fungal species and sequencing of Aspergillus species. Ranges of total bacterial count of flour, dough and bread samples collected from breads were between 2.83±0.99 and 6.43±1.12 log cfu/g. Flour, dough and bread samples collected from bulk breads had the higher counts of total bacteria than those of flat breads. Ranges of mold count of flour, dough and bread samples collected from breads were between 0.00±0.00 and 1.63±0.63 log cfu/g. Flour, dough and bread samples collected from flat breads had the higher counts of mold than those of bulk breads.

Distribution of Penicillium, Cladosporium, Mucor, Aspergillus, Alternaria, Caininghamella, Hylane, Rhizopus, Epicoccum and Syncephalestrum amongst detected fungal colonies were 24.40%, 20.10%, 20.10%, 19.00%, 3.80%, 3.80%, 3.80%, 2.70%, 1.00% and 1.00%, respectively. Sequencing of isolated Aspergillus genus revealed that all strains were related to flavus, oryzea, terreus and nijer species. Detected total count and mold count in studied bread samples were lower than limit standards announced by Institute of Standards and Industrial Research of Iran. However, considering the high consumption rate of these kinds of food samples among Iranian people, severe surveillance programs should perform to inhibit from their bacterial and fungal contamination.

Keywords: Total bacterial count, Fungal species, Identification, Bakery products.
toxicity[9]. Aflatoxins which are mainly produced by *Aspergillus* species, especially *Aspergillus flavus* (*A. flavus*) and *A. parasiticus* are the most clinically important mycotoxins[14]. Aflatoxins are classified as group 1A potential human carcinogens by an International Agency of Research on Cancer[14]. Diverse surveys have been conducted on the role of animal species, particularly their resources such as milk and meat as a vector of fungal transmission to human population [9, 14].

Mycotoxins are resistant to high cooking temperatures and unfavorable environmental conditions, and are often found in processed and cooked foods[15-17] which make them important from the public health prospective. Thus, it is important to assess the fungal population of breads and bakery product to ensure their hygienic qualities. Several researches have been conducted on fungal contamination of flour and breads but they have almost been concentrated on fungal counts [18-20]. In the other hand, there were no previously published data on determination of fungal species in flour, bread and dough samples. Conventional identification of fungal species was based on specified and time-consuming morphological characteristics on culture media[21]. However, rapid, safe and sensitive detection of fungal species using polymerase chain reaction (PCR)-based amplification of certain target genes has been developed as accurate and valid alternative method [14, 22].

Institute of Standards and Industrial Research of Iran (ISIRI) has been determined some standard limits for fungal and especially mold contamination of bread and bakery products. Though, several investigations have been reported fungal contamination of flour and bakery products higher than announced standard limits of ISIRI[3, 23-25]. Moreover, higher distribution of *Aspergillus* species has also been reported in Iran[12, 25, 26]. Furthermore, there were no PCR-based identification of fungal population of breads and other bakery product in Iran [23-26]. Therefore, the present research was done to assess the fungal population of bread, flour and dough samples collected from two major types of breads (flat and bulk breads) and mold species identification using PCR and sequencing analysis.

### Material and Methods

#### Sampling

The present descriptive cross-sectional study was carried out from October 2017 to March 2018 in the Isfahan city, Iran. Two-hundred and forty bakery samples including flour (n=80), dough (n=80), and bread (n=80) were randomly collected from 20 local bakeries of the Isfahan city, Iran. Samples were collected from two major types of breads (flat and bulk breads). Samples were immediately transferred to Food Safety Research Center of the Isfahan University of Medical Sciences, Isfahan, Iran in cooler with ice-packs.

#### Mold count

Ten grams of each sample was added to 90 ml of Ringer’s solution and shaken for approximately 10 min. Then, serial dilution was done to -1000 fold. Aliquots consisting of 1 ml of each dilution were spilled into the plate and poured by yeast-extract-glucose chloramphenicol agar(YGCA, Merck, Darmstadt, Germany, pH=6.6±0.2). The arrangement of medium was (g/liter): yeast extract 5.0, D (+) glucose 20.0, chloramphenicol 0.1, agar-agar 14.9. After 5 days incubation at 25°C, mold colonies were counted according to the method described previously [27].

#### Total count

Ten grams of each sample was added to 90 ml of Ringer’s solution and shaken for approximately 10 min. Then, serial dilution was done to -1000 fold. Aliquots consisting of 1 ml of each dilution were spilled into the plate and poured by plate count agar(PCA, Merck, Darmstadt, Germany, pH=7.0±0.2). The composition of medium was (g/liter): peptone from casein 5.0, yeast extract 2.5, D(+)glucose 1.0, agar-agar 14.0. After 3 days incubation at 30°C, colonies were counted according to the method described previously [27].

#### Isolation and Identification of fungi

Species of fungal strains were further identified using the Malt Extract Agar (MEA, Merck, Darmstadt, Germany) media. Cultures were incubated at 25°C for 7 days and then the individual genera of molds were taxonomically identified on the basis of their macroscopic and microscopic morphology according to method described previously [28-30].
DNA extraction

Genomic DNA was extracted from the fungal colonies using the phenol-chloroform DNA extraction protocol. Briefly, 10 µL spores of mouldes were added to 300 µl of lysis buffer (100 mmol Tris, 20 mmol EDTA, 100 mmol NaCl, 2% SDS). After grinding for 1 minute, 300 µl of phenol-chloroform solution was added and centrifuged at 5000 g for 5 min. The aqueous phase was moved to a new tube and 300 µl phenol-chloroform was added and centrifuged at 5000 g for 5 min. Nucleic acids in the aqueous phases were precipitated with 0.1 volume of sodium acetate 3 M and equal volume of isopropanol, incubating at -20 °C for 10 min. After centrifugation at 10000 g for 10 min, 300 µl of 70% ethanol was added to the precipitates and centrifuged for 5 min. To conclude with the supernatant discarded and 50 µl of deionized distilled water were added to the precipitate and the pellets of DNA were kept at -20 °C[31]. Extracted DNA samples were subjected to quantification by NanoDrop device (NanoDrop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel) and purity checking (A260/A280).

The universal fungal primers

Bt2α (5’GGTAACCAAATCGGTGCTGCTTTC3’) and Bt2b (5’ACCCTCAGTGTA GTGACCTTGGC 3’) (650 bp) primers were used for PCR amplification of Beta-tubulin gene (Kamari et al., 2017). PCR mixture contained 5 Ml of extracted DNA, 25 pmol of each Bt2α and Bt2b primers, 400 µmol dNTPs, 2.5 µl buffer, 1.5 mmol mgcl2, 2.5 U of Taq polymerase in a final volume of 25 µl. The PCR amplification was done as follow: denaturation of DNA at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 1 min, with a final extension phase at 72°C for 6 min [31]. Seven microliters of each PCR product with three microliters of loading buffer were run onto 1.5% agarose gel and electrophoresed in Tris-borate-EDTA buffer. Gels have been moved to the transilluminator machine and taken photos of bands.

Sequencing

The amplicons were purified and cycle sequencing reactions were performed in forwarding direction. The raw nucleotide sequencing was analyzed with MEGA 4 software(MEGA version 4). Resulting sequences of isolates were evaluated using NCBI BLAST searches for fungal sequences existing in DNA databases (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

Data obtained from the experiments were transferred to Excel software. SPSS/21.0 software was applied for statistical analysis. Data were analyzed using Univariate Analysis Of Variance (ANOVA) and one sample T-test. Numerical meaning was regarded at a P value < 0.0005.

Results

A total of 240 cereal samples including flour, dough, and bread were collected from flat and bulk breads and studied for microbial and fungal contamination and identification of fungal species. Table 1 represents the total and mold counts of flour, dough, and bread samples collected from flat and bulk breads. Total bacterial count of flour, dough, and bread samples collected from flat breads had ranges between 2.8328±0.9892 and 6.2153±0.7817 log cfu/g. Additionally, Total bacterial count of flour, dough and bread samples collected from bulk breads had ranges

| Types of bread samples | Mean microbial counts (Log cfu/g ± standard deviation (SD)) |
|------------------------|-------------------------------------------------------------|
|                        | Total count in PCA | Mold count in YGC |
| Flat bread             |                 |                   |
| Flour                  | 3.8623±1.0556a   | 1.6271±0.6283a    |
| Dough                  | 6.2153±0.7817b   | 0.0000±0.0000b    |
| Bread                  | 2.8328±0.9892c   | 0.1010±0.4459b    |
| Bulk bread             |                 |                   |
| Flour                  | 4.0659±0.7594a   | 1.5596±0.9203a    |
| Dough                  | 6.4335±1.1192b   | 0.0000±0.0000b    |
| Bread                  | 3.1478±1.0132c   | 0.0000±0.0000b    |

*Dissimilar letters in each column shows statistically significant differences about P<0.0005.
between 3.1478±1.0132 and 6.4335±1.1192 log cfu/g. Flour, dough and bread samples collected from bulk breads had the higher counts of total bacteria than those of flat breads. Otherwise, dough samples in both types of breads had the higher rate of total bacterial contamination, while bread had the lowest. Mold count of flour, dough and bread samples collected from flat breads had ranges between 0.0000±0.00000 and 1.6271±0.6283 log cfu/g. Moreover, mold count of flour, dough and bread samples collected from bulk breads had ranges between 0.0000±0.0000 and 1.5596±0.9203 log cfu/g. Flour, dough and bread samples collected from flat breads had the higher counts of mold than those of bulk breads. Otherwise, flour samples in both types of breads had the higher rate of mold contamination, while dough had the lowest. Statistically significant differences were found between the total bacterial count and mold count of bread samples of flat breads and bulk breads (P<0.0005).

Table 2 represents the distribution of different species of fungi amongst the colonies detected in flour, dough and bread samples collected from flat and bulky breads. All of the identified fungi species were found in flour samples except *Epicoccum* which was isolated from colonies detected in bread samples. *Penicillium* had the highest distribution (24.29%) amongst studied colonies, while *Epicoccum* had the lowest (1.43%). Additionally, 19.29% of detected fungal colonies were related to *Aspergillus* species.

Sequencing analysis of agarose gel electrophoresis of each PCR product in comparison with fungal sequences existing in DNA databases showed that *A. flavus*, *A. oryzea*, *A. terreus*, and *A. niger* were the main genus amongst all isolated *Aspergillus* isolates. Total incidence of *A. flavus*, *A. oryzea*, *A. niger* and *A. terreus* species amongst the studied flour samples were 55.55%, 18.52%, 18.52% and 7.41%, respectively.

**Discussion**

Due to the low content of activated water in cereals, breads and other types of bakery products, their contamination throughout the harvesting, storage and preparation were accompanied with fungi and especially molds. Some types of these mold are responsible for production of important mycotoxins with important complications on human health. Thus, it is important to assess the fungal population and mold species identification in the bread, flour and dough samples as a highly consumed food stuffs not only among Iranian people but also among people of all sites of the world.

The present research was done to assess the fungal population and species identification of molds in bread, flour and dough samples collected from two different types of breads (flat and bulk). According to the data, 35.80% of samples had a total count of microorganisms higher than the maximum accepted levels announced by ISIRI (standard level=5 log_{10} cfu/g). Additionally, all of

**TABLE 2. Distribution of fungal species amongst the detected colonies in flour, dough and bread samples collected from flat and bulky breads.**

| Mold species  | Source    | n   | (%) Distribution |
|---------------|-----------|-----|------------------|
| Penicillium   | Flour     | 34  | 24.29            |
| Cladosporium  | Flour     | 28  | 20               |
| Mucor         | Flour     | 28  | 20               |
| Aspergillus   | Flour     | 27  | 19.29            |
| Alternaria    | Flour     | 5   | 3.57             |
| Canninghamella| Flour     | 5   | 3.57             |
| Hyaline       | Flour     | 5   | 3.57             |
| Rhizopus      | Flour     | 3   | 2.14             |
| Epicoccum     | Bread     | 2   | 1.43             |
| Syncephalestrum| Flour    | 3   | 2.14             |
| Total         |           | 140 | 100              |

*Egypt. J. Vet. Sci. Vol. 51, No.2 (2020)*
our studied samples had acceptance counts of fungi compared to limit standards announced by the ISIRI (standard level=3.698 log_{10} cfu/g). However, several investigations were reported fungal contamination higher than permissible maximum levels[23-25]. Total mold count obtained in the present research was similar to the those reported by previous Iranian investigation conducted on northwest of Iran[3]. Our findings showed that there were no detectable counts for molds in dough samples. This finding is mainly due to the higher activity of yeast in dough samples which caused inhibition from the growth of molds. The highest mean amounts of total count was found in dough samples, while the highest mean amounts of mold count was found in flour samples. Thus, our finding showed that thermal processing decreased the microbial population of bakery products. This finding was similar to the results of previously published data[32-34]. Therefore, the microbial contamination of bread samples may occur after the baking procedure of breads. It may be occur due to the hand manipulation, cutting with a knife and even unfavorable keeping conditions[30]. No statistically significant difference was obtained for the numbers of molds and also total counts between flat and bulk bread samples.

Majority of identified fungal species had zoonotic importance and can transfer from infected animals and even birds into the human population. Thus, they mainly have veterinary impacts. Additionally, some kinds of studied cereals, especially corrupted bread samples, were mainly used for animal feeding. Unfortunately, ranchers were used from these kinds of cereals for feeding of dairy cows, sheep and goats in some parts of Iran. Thus, fungal contaminants will transfer to human population through resources such as milk and meat to human population. Thus, detection of the above mentioned fungal species in bread samples has veterinary-based importance. Feeding of animal species with fungal contaminated bread may cause severe abortion and also mastitic in animal species, especially dairy livestock.

Different species of mold such as Penicillium, Cladosporium, Mucor, Aspergillus, Alternaria, Canninghamella, Hyaline, Rhizopus, Epicoccum and Syncphalestrum were found in studied samples of our research. Penicillium was the most frequently identified mold in studied samples. This finding is also similar to results of previous investigations[35]. Similar fungal population was also reported in some previously reported papers[21, 23-26, 36, 37]. Otherwise, high prevalence of Fusarium species has been reported in cereals and wheat flour [5, 12, 35, 38, 39]which was dissimilar to our results. The differences found in the types of molds reported in various investigations is may be due to the differences in types of samples, method of sampling and weather and climate of sampling places. Detected fungi in the present investigation were reported previously as the most important causes of severe clinical implications[40, 41]. Alternaria is a ubiquitous pathogen with high ability to produce toxin [42-45]. However, it had a low frequency in the samples of our investigation. The existence of Syncphalestrum amongst the identified molds in studied samples is so important due to its ability to produce particular mycotoxins. This finding is in agreement with the results of fungal contamination of wheat reported by recent survey[24]. Mucor is another important identified fungi in studied samples. It is responsible for stone formation in human urinary tract by synthesize oxalic acid and then binding of oxalates to calcium and other minerals[46]. Thus, further studies should address in order to control the presence of Mucor in bakery products.

Our results in PCR sequencing of fungal strains is similar to those reported by Riba et al conducted on Algerian wheat [39]. A. flavus, A. niger and A. terreus are common fungi in studied bread samples. A. flavus can produce aflatoxin [47, 48]. Additionally, A. niger can produce ochratoxin [49, 50]. Furthermore, A. terreus is resistant to antifungal drugs [51]. Thus, their high prevalence in the fungal population of bread samples collected from Isfahan, Iran pose an important public health threat regarding the consumption of these products[52]. Foodborne pathogens have boost clinical and microbial importance in Iran. Thus, detection of fungal species in cereals samples has a high health-related and food hygienic importance regarding the consumption of this foodstuffs by human and in some cases like corrupted bread samples by animal species.

Conclusions

Inconclusion, high microbial and mold counts in the flour, dough and bread samples collected from Isfahan city, Iran were found. Bulk breads
had the higher counts of total bacteria than flat breads. Dough samples had the higher rate of total bacterial contamination, while bread had the lowest. Flat breads had the higher counts of mold than bulk breads. Otherwise, flour samples had the highest rate of mold contamination, while dough had the lowest. All studied bread samples had the lower mold count than accepted levels announced by ISIRI, while 35.80% of samples had a total count of microorganisms higher than the maximum accepted levels. Penicillium, Cladosporium, Mucor and Aspergillus were the most commonly identified fungi amongst the bread samples. Considering the high ability of some of the identified fungi in mycotoxin production, several surveillance programs should perform to prevent from fungal contamination of bread and bakery products. In keeping with the high distribution of total microorganisms and molds in bread samples, further researches should perform to find the exact routes of contamination and also other microbiological and health related aspects of presence of fungi in bread and its products.

Acknowledgements
Authors would like to express special gratitude to Dr. Mohammad Kazerooni the manager of the Isfahan Atlas flour company for his technical supports, useful comments, and friendly advice during this research.

Ethical consideration
The research was permitted by the Moral Council of Research of the Isfahan University of Medical Sciences, Isfahan, Iran (96-2020). Confirmation of the current investigation and the certificates associated with sampling procedure were permitted by Dr. Masoud Sami and Dr. Maryam Mirlohi.

Conflict of interest: The authors declared that no conflict of interest.

Funding statement: Self funding

References
1. Salehifar, M. Shahedi, M. Effects of oat flour on dough rheology, texture and organoleptic properties of taftoon bread. Journal of Agricultural Science and Technology, 9,227-234 (2010).
2. Alami, A., Banoorkar, S., Rostamiyan, T., Asadzadeh, S.N. Morteza, M.M., Quality assessment of traditional breads in Gonabad bakeries, Iran. Journal of Research and Health. 4 (3), 834-840 (2014).
3. Asadzadeh, J., Teymori, R., Ghazanfarirad, N., Fakhernia, M., Haghhighat-Afshar, N., Blouki, M., Kheiri, A., Hassanazadazar, H. and Bahmani, M. Fungal contamination of produced wheat flour in West Azerbaijan, northwest of Iran. Asian Pacific Journal of Tropical Disease,4,S836-S839 (2014).
4. Menteş, Ö., Bakkalbaşı, E. and Erçan, R. Effect of the use of ground flaxseed on quality and chemical composition of bread. Food Science and Technology International. 14(4),299-306 (2008).
5. Berghofer, L.K., Hocking, A.D., Miskelly, D. and Jansson, E. Microbiology of wheat and flour milling in Australia. International journal of food microbiology, 85(1-2),137-149 (2003).
6. Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B. and Givskov, M., Food spoilage—interactions between food spoilage bacteria. International Journal of Food Microbiology, 78(1-2),79-97 (2002).
7. Antony-Babu, S. and Singleton, I., Effects of ozone exposure on the xerophilic fungus, Eurotium amstelodami IS-SAB-01, isolated from naan bread. International Journal of Food Microbiology. 144(3),331-336 (2011).
8. Faramarzi, T., Jonidi Jafari, A., Dehghani, S., Mirzabeygi, M., Naseh, M. and Rahbar Arasteh, H. A survey on Bacterial Contamination of Food Supply in the West of Tehran. Journal of Fasa University of Medical Sciences, 2(1),11-18 (2012).
9. Gerez, C.L., Torino, M.I., Rollán, G. and De Valdez, G.F., Prevention of bread mould spoilage by using lactic acid bacteria with antifungal properties. Food Control, 20(2),144-148 (2009).
10. Khoshakhlagh, K., Hamdami, N., Shahedi, M. and Le-Bail, A., Quality and microbial characteristics of part-baked Sangak bread packaged in modified atmosphere during storage. Journal of Cereal Science, 60(1),42-47 (2014).
11. Nguyen, P.-A., Strub, C., Fontana, A. and Schorr-Galindo, S., Crop molds and mycotoxins: Alternative management using biocontrol. Biological Control, 104,10-27 (2017).
12. Okhovvat, S. and Zakeri, Z., Identification of fungal diseases associated with imported wheat in Iranian silos. Communications in Agricultural and Applied Biological Sciences, 68(4 Pt B),533-535 (2003).
13. Škrbić, B., Živančev, J., Đurišić-Mladenović, N. and Godula, M., Principal mycotoxins in wheat flour from the Serbian market: Levels and assessment of the exposure by wheat-based products. Food Control,25(1),389-396 (2012).
14. Sardiñas, N., Vázquez, C., Gil-Serna, J., González-Jaén, M.T. and Patiño, B., Specific detection and quantification of Aspergillus flavus and Aspergillus parasiticus in wheat flour by SYBR® Green quantitative PCR. *International Journal of Food Microbiology*.*145*(1),121-125 (2011).

15. Boudra, H., Le Bars, P. and Le Bars, J., Thermostability of Ochratoxin A in wheat under two moisture conditions. *Applied and Environmental Microbiology*.61(3),1156-1158 (1995).

16. Jackson, L.S., Hlywka, J.J., Senthil, K.R. and Bullerman, L.B., Effects of thermal processing on the stability of fumonisin B2 in an aqueous system. *Journal of Agricultural and Food Chemistry*.44(8),1984-1986 (1996).

17. Ryu, D., Hanna, M.A., Eskridge, K.M. and Bullerman, L.B., Heat stability of zearalenone in an aqueous buffered model system. *Journal of Agricultural and Food Chemistry*.51(6),1746-1748 (2003).

18. Eyles, M. and Hocking, A., The microbiological status of Australian flour and the effects of milling procedures on the microflora of wheat and flour. *AGRIS since*, 41(4),407-408 (2013).

19. Hesseltine, C. and Graves, R., Microbiology of flours. *Economic Botany*, 20(2),156-168 (1966).

20. Rogers, R. and Hesseltime, C., Microflora of wheat and wheat flour from six areas of the United States. *Cereal Chem.*, 55(6),889-898 (1978).

21. Weidenbörmer, M., Wieczorek, C., Appel, S. and Kunz, B., Whole wheat and white wheat flour-the mycobiota and potential mycotoxins. *Food Microbiology*.17(1),103-107 (2000).

22. Rodríguez, A., Rodríguez, M., Martín, A., Nuñez, F. and Córdoba, J.J., Evaluation of hazard of aflatoxin B1, ochratoxin A and patulin production in dry-cured ham and early detection of producing moulds by qPCR. *Food Control*, 27(1),118-126 (2012).

23. Ebrahimzadeh, A., Mohammadzadeh, F. and Salimi, A., Prevalence of fungal contamination of flour in zahedan bakeries in 2013. *Medical Journal of Mashhad University of Medical Sciences*, 57, 705-710 (2014).

24. Kazemi, A., Razavie, S., Rezaazadeh, A., Pirzeh, L., Hosseini, M., Vahed-Jaberi, M., Ghaemmaghami, S. and Jafari, A., Fungal contamination of flours in bakeries of tabriz city. *Medical Journal of Mashhad University of Medical Sciences*, 50, 411-418 (2018).

25. Sadeghi, E., Mesgarof, H., Sharifi, K., Almasi, A., Bohluli, S. and Meskini, H., Study of microbiological quality of flour produced in Kermanshah and Ilam factories. *Journal of Nutrition Sciences & Food Technology*, 8(4),13-20(2014).

26. Sarafi, O., Faezi Ghasemi, M. and Chaeiechi Nosrati, A., Isolation and characterization of toxicocenic fungi strains from wheat and corn used in Kerman city. *Journal of Microbial World* 8(4),330-336 (2016).

27. Organization, I.N.S., Cereal product and pulses, flour and soy protein-Microbial specification and test methods, 2393. (2013).

28. Ciardo, D.E., Schär, G., Althegg, M., Böttger, E.C. and Bosshard, P.P., Identification of moulds in the diagnostic laboratory—an algorithm implementing molecular and phenotypic methods. *Diagnostic Microbiology and Infectious Disease*, 59(1),49-60 (2007).

29. Gams, W., More dematiaceous Hyphomycetes. *European Journal of Plant Pathology*. 83(2),90-90 (1977).

30. Riba, A., Bouras, N., Mokrane, S., Mathieu, F., Lebrhi, A. and Sabaou, N., Aspergillus section Flavi and aflatoxins in Algerian wheat and derived products. *Food and Chemical Toxicology*.48(10),2772-2777 (2010).

31. Kamari, A., Sepahvand, A. and Mohammadi, R., Isolation and molecular characterization of cryprococcus species isolated from pigeon nests and Eucalyptus trees. *Current Media Mycology*.3(2),20-25 (2017).

32. Ahn, J., Balasubramaniam, V. and Yousef, A., Inactivation kinetics of selected aerobic and anaerobic bacterial spores by pressure-assisted thermal processing. *International Journal of Food Microbiology*.113(3),321-329 (2007).

33. Krebbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M. and van den Berg, R.W., Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Science & Emerging Technologies*.4(4),377-385 (2003).

34. Rajan, S., Pandrangi, S., Balasubramaniam, V. and Yousef, A.E., Inactivation of Bacillus stearothermophilus spores in egg patties by pressure-assisted thermal processing. *LWT-Food Science and Technology*. 39(8),844-851 (2006).
35. Kachuei, R., Hossein, Y.M., Susan, R., Abdolamir, A., Naser, S., Farideh, Z. and Fatemeh, K.Y., Investigation of stored wheat mycoflora, reporting the Fusarium cf. langsethiae in three provinces of Iran during 2007. *Annals of Microbiology, 59*(2),383-390 (2009).

36. Bu’Lock, J., Mycotoxins as secondary metabolites. In *The Biosynthesis of Mycotoxins: A Study in Secondary Metabolism*. Academic Press New York: 1-16 (1980).

37. Lacey, J., The microbiology of cereal grains from areas of Iran with a high incidence of oesophageal cancer. *Journal of Stored Products Research*. 24(1),39-50 (1988).

38. Al-Defiery, M.E. and Merjan, A.F., Mycoflora of mold contamination in wheatflour and storage wheat flour. *Mesop. Environ. J.*, 1(2),18-25 (2015).

39. Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A. and Sabaou, N., Mycoflora and ochratoxin A producing strains of Aspergillus in Algerian wheat. *International Journal of Food Microbiology, 122*(1-2),85-92 (2008).

40. Curtis, L., Lieberman, A., Stark, M., Rea, W. and Vetter, M., Adverse health effects of indoor molds. *Journal of Nutritional & Environmental Medicine, 14*(3),261-274 (2004).

41. Fung, F. and Hughson, W.G., Health effects of indoor fungal bioaerosol exposure. *Applied Occupational and Environmental Hygiene, 18*(7),535-544 (2003).

42. Bottalico, A. and Logrieco, A., Toxigenic Alternaria species of economic importance. In *Mycotoxins in Agriculture and Food Safety*. CRC Press: 83-126. (1998)

43. Ostry, V., Alternaria mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. *World Mycotoxin Journal, 1*(2),175-188 (2008).

44. Scott, P.M., Analysis of agricultural commodities and foods for Alternaria mycotoxins. *Journal of AOAC International, 84*(6),1809-1817 (2001).

45. Yu, W., Yu, F.-Y., Undersander, D.J. and Chu, F.S., Immunoassays of selected mycotoxins in hay, silage and mixed feed. *Food and Agricultural Immunology, 11*(4),307-319 (1999).

46. Gontzea, I. and Sutzescu, P. Book: *Natural antinutritive substances in foodstuffs and forages*, pp.viii+184 pp. (1968).

47. Davis, N.D., Diener, U. and Eldridge, D., Production of aflatoxins B1 and G1 by Aspergillus flavus in a semisynthetic medium. *Applied Microbiology, 14*(3),378-380 (1966).

48. Hedayati, M., Pasqualotto, A., Warn, P., Bowyer, P. and Denning, D., Aspergillus flavus: human pathogen, allergen and mycotoxin producer. *Microbiology, 153*(6),1677-1692 (2007).

49. Abarca, M., Bragulat, M., Castella, G. and Cabanes, F., Ochratoxin A production by strains of Aspergillus niger var. niger. *Applied and Environmental Microbiology, 60*(7),2650-2652 (1994).

50. Schuster, E., Dunn-Coleman, N., Frisvad, J. and Van Dijck, P., On the safety of Aspergillus niger—a review. *Applied Microbiology and Biotechnology, 59*(4-5),426-435 (2002).

51. Eliopoulos, G.M., Perea, S. and Patterson, T.F., Antifungal resistance in pathogenic fungi. *Clinical Infectious Diseases, 35*(9),1073-1080 (2002).

52. Steinbach, W.J., Benjamin Jr, D.K., Kontoyiannis, D.P., Perfect, J.R., Lutsar, I., Marr, K.A., Lionakis, M.S., Torres, H.A., Jafri, H. and Walsh, T.J., Infections due to Aspergillus terreus: a multicenter retrospective analysis of 83 cases. *Clinical Infectious Diseases, 39*(2),192-198 (2004).