PHYSICOCHEMICAL PROPERTIES AND AFLATOXINS ASSESSMENT IN COMMERCIALLY AVAILABLE WHEAT FLOUR

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ABSTRACT

Aim: Physicochemical properties and aflatoxins level were determined in wheat flour collected from flour mills (FM), shops (SH), houses (HO) and tandoors (TA).

Methods: Physicochemical properties (moisture content, ash, fat, fiber, protein and Nitrogen free extract) were tested by the standard methods. Aflatoxins level was determined by standard protocol; using high performance liquid chromatography (HPLC) equipped with UV/VIS detector.

Results: Aflatoxins were positively correlated with ash, fiber, protein and moisture content whereas fat and nitrogen free extract were negatively correlated. Furthermore, proximate analysis demonstrated moisture content ranges from 7.02 -13.88%, ash 0.51 - 0.83%, fat 0.91 - 1.6%, fiber 0.41 - 0.65%, protein 9.06 - 13.89% and NFE 70.31 - 80.98% respectively. Samples from houses were found severely contaminated with average aflatoxins level; 14.90µg/kg followed by 18.40, 12.55 and 15.67 µg/kg in FM, SH and TA respectively.

Conclusion: It can be concluded that high moisture content in wheat flour may be associated with aflatoxin contamination.

Keywords: Aflatoxins, wheat flour, HPLC, Physicochemical analysis

INTRODUCTION

Wheat (Triticum aestivum) is common food for man and it constitutes major source for energy in the diet in developing countries, especially in Pakistan. It is consumed as a primary source of proteins and carbohydrates and also a good source of minerals. It is the staple diet of

Pakistani population and is mostly used in the form of chapatti and other wheat based products [1]. Pakistani population consumes 80% of the total cereal intake in the form of wheat; hence wheat is the foremost and economical source of energy [2]. It is the cheapest and principal source of energy and protein for the inhabitants of Pakistan. The annual wheat production stood at 21.74 million tonnes during the year 2007-08 [3].Almost 80% of this is consumed in the form flour for making of flat breads locally known as chapattis, rotis and naan. It is a staple food and contributes 68-75% of total food intake in daily diet for the people of Pakistan [4]. Pakistan is one of the largest growers of wheat; it exports wheat to nearby countries after fulfilling its requirements. Therefore it is necessary to ensure the quality and safety of wheat being exported to other countries.

High content of gluten help in making bread or chapatti and create elastic toughness that holds its shape well once baked staple. Wheat is the most favorite cereals used for the production of flour due to the high content of gluten [5]. It provides upwards of 60 percent of the protein and carbohydrate in the average diet [6, 7]. Moisture in flour is very important factor and it's normally ranges from 11–14 percent. When moisture content rises above 14 percent, flour is susceptible to fungus and mold growth, flavor changes, enzyme activity, and insect infestation. Ramesh et al relates the growth of aflatoxins with inappropriate post-harvest technology and deprived storage conditions [8]. Another study reported that storage of baby's food at high level of humidity for seven days increased the fungal growth without affecting the toxin level [9].

Fungi are organisms that are found everywhere in nature and are major spoilage agents of food and feed stuffs. Fungal flora not only spoils lot of food stuff including grains but also produce toxins depending upon the environmental conditions favorable for their growth. Growth of fungi on such stuffs results in the production of fungal poisonous secondary metabolites such as mycotoxins which are associated with serious health disorders, characterized by nephrotoxity, immunotoxic, teratogenic and mutagenic, which can cause acute and chronic in man and animals ranging from central nervous system disorders, cardio vascular problems and pulmonary system disorders to death [10]. Ghasemi-Kebria et al. assessed aflatoxins in wheat flour in high and low esophageal cancer risk areas and reported a positive relationship in aflatoxin, wheat flour and esophageal cancer [11]. Tropical and subtropical regions of the world are more adversely affected with aflatoxins, where climatic conditions of temperature and relative humidity favor the growth of Aspergillus flavus and A. parisiticus. In developing countries, poor conditions of

food storage is of great concern with fungal growth and its metabolites (mycotoxins) and this causes serious health hazards, threats to food security and international trade. Therefore fungal and mycotoxin contamination should be monitored periodically in order to meet the international regulatory standards. In climate like Pakistan, the shelf life of flour is a serious problem and due to weather conditions, it is inevitable to explore proper moisture content to overcome existing dilemma, the present study was conducted to investigate the physicochemical properties and aflatoxin level in wheat flour produced by certain species of molds.

MATERIALS AND METHODS

Sampling

Wheat flour samples were collected in the months of August and September, from ten (10) flour mills (FM), shops (SH), houses (HO) and tandoors (TA) each, from Peshawar city, Khyber Pakhtunkhwa (KP), Pakistan, according to standard procedure [12]. Specimens were packed in sterile polythene bags and transported to the laboratory of Food Science Division, Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar stored at 4°C for further studies.

Proximate analyses of wheat flour

Proximate analyses of collected specimens were conducted for moisture content, ash, fat, fiber and protein according to the standard method [13]. Nitrogen free extract (NFE) was calculated by subtracting all the percentage values from 100.

Moisture and Ash determination

Moisture and ash were determined through oven dehydration method [13].

Crude fat determination

Crude fat was determined through soxhlet apparatus according to method no. 30-10.01 [13].

Crude fiber determination

Crude fiber was determined through method no. 32-10.01 [13].

Crude protein determination

Nitrogen present in each sample was estimated by using Kjeldahl method as described by method no. 46-10.01 [13].

Nitrogen free extract

NFE (represent the digestable carbohydrates) was calculated by subtracting all the percentages including moisture, ash, crude fat, crude protein and crude fiber from 100 [14].

Determination of aflatoxins

Aflatoxins level was determined following the standard protocol as previously described [15], using high performance liquid chromatography (HPLC). The instrument was equipped with reversed phase C18 column (Brownlee Analytical), Isocretic pump (Perkin Elmer series 200) and UV/VIS detector. HPLC peak quantification was carried out by using Total Chrom software (Version 6.3.2). All the solvents used for analysis were of analytical grade.

The elution solvent system was used as water/methanol/acetonitrile (50/40/10, v/v/v) [16]. Ten microliter (10µl) elute was injected to HPLC. The flow rate was adjusted to 1ml per minute and detected through UV detector (365nm). The response was recorded in the form of peaks and compared to the peaks obtained from the standard. The results were calculated through software.

Statistical Analysis

Statistical analysis was carried out by using Statistical software Statistix® 1.8. Least Significant Difference (LSD) test was employed to separate means at 5% level of probability.

RESULTS AND DISCUSSIONS

Proximate composition of wheat flour

The current results obtained from proximate analysis were in accordance with earlier finding [18] and demonstrated moisture, ash, fat, fiber, protein and nitrogen free extract 7%, 1.50%, 3.60%, 4.50%, 13.9% and 69.50% respectively.

Moisture and Ash Contents

Average moisture contents (%) of wheat flour collected from FM, SH, HO and TA was 9.52, 9.71, 9.55 and 8.03% respectively (Fig. 1). The highest moisture content was observed for FM sample (6) which was 13.88 % while the lowest value was observed for TA sample (9) which was 7.03 %. The results obtained from this study are in relevance to the earlier findings [19] and they reported 8.83% moisture content in wheat flour. Study on the proximate composition of wheat flour and soy flour composite breads showed an increase in moisture content. Increase in moisture content has been associated with increase in fiber content [20, 21, 22]. High moisture content has been associated with short shelf life of composite breads as they encourage microbial proliferation that lead to spoilage [23].

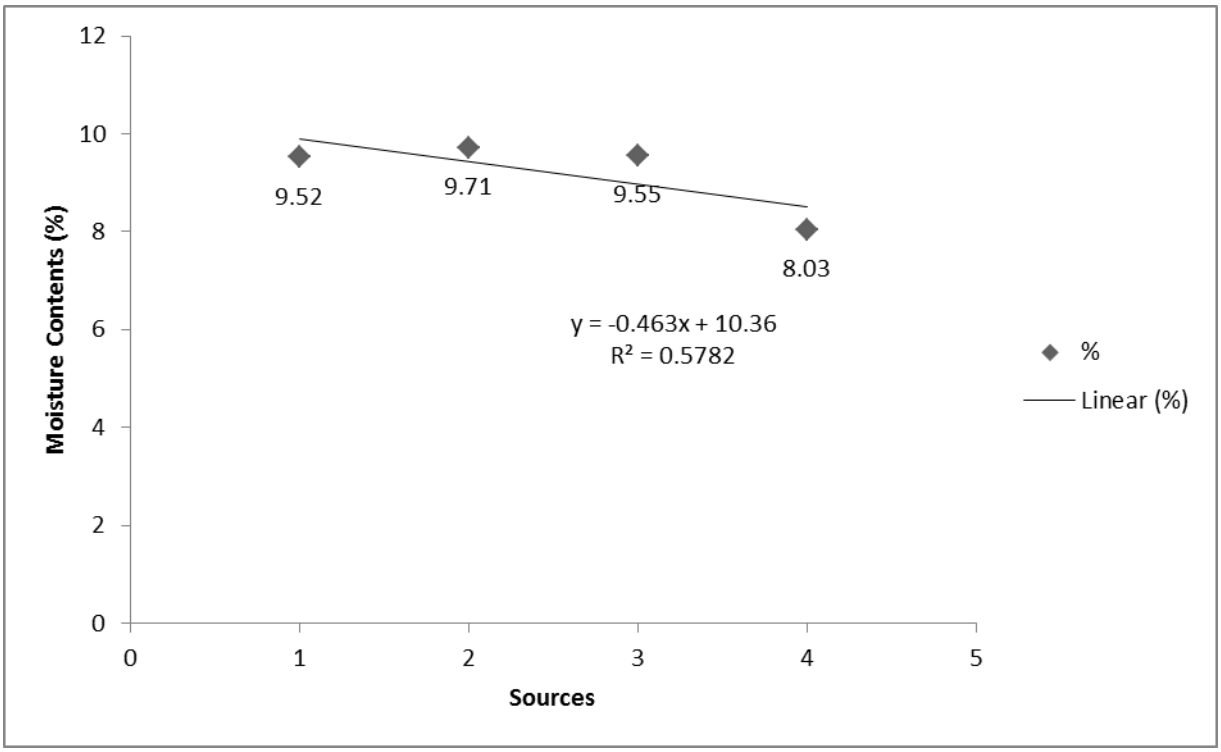


Fig. 1. Moisture content (%) in wheat flour samples collected from different sources, where 1 (FM), 2 (SH), 3(HO) and 4 (TA).

Results showed the average ash contents 0.64, 0.57, 0.59 and 0.67% for FM, SH, HO and TA respectively (Fig. 2). The highest value observed was for TA sample (6) which was 0.86% and lowest value was observed for FM, SH sample (3) and HO sample (5) which was 0.51%. Statistical analysis showed that the means of treatment TA had a significant effect (p=0.02) on ash content in wheat flour. The results obtained from the study are in close conformity to the earlier study [24] and they reported a range of 0.52% - 0.67% ash content in commercial wheat flour.

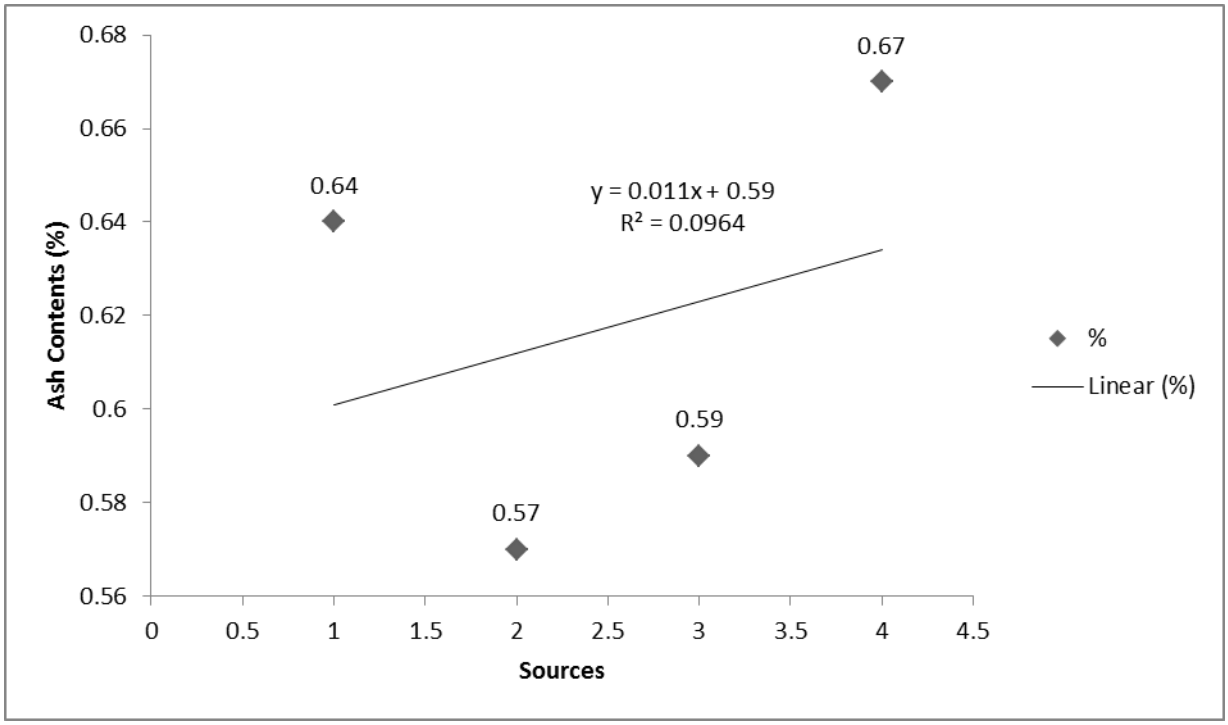


Fig. 2. Ash contents (%) in wheat flour samples collected from different sources, where 1 (FM), 2 (SH), 3(HO) and 4 (TA).

Fat Content

Results showed 0.95, 1.43, 1.19 and 1.12 % average fat contents for FM, SH, HO and TA respectively (Fig. 3). Highest value was observed for SH sample (10) and TA sample (9) which was 1.6% while lowest value was observed for FM sample (3, 4) and HO sample (5) which was 0.91%. Statistical analysis of the obtained data showed that treatment SH had a significant effect (p= 0.00) on the fat content of wheat flour. The results obtained from the study are similar to the earlier published results [24]. They reported a range of 0.94% - 1.15% fat content in commercial

wheat flour. The proximate composition of pita bread was determined and wheat flour used in the preparation of major types of bread in Kuwait. A significant loss in fat was noted in bread as compared to the flour [25].

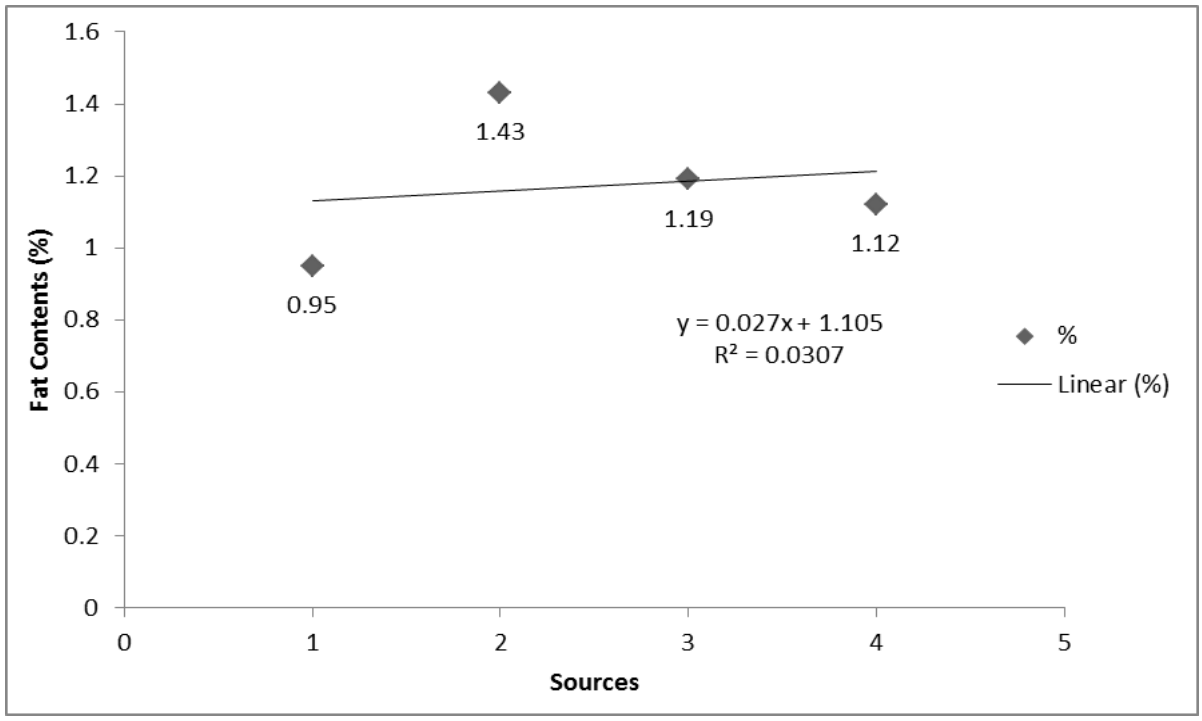


Fig. 3. Fat content (%) in wheat flour samples collected from different sources, where 1 (FM), 2 (SH), 3(HO) and 4 (TA).

Fiber Content

Highest value for fiber content was observed for FM sample (1) i.e. 0.65% while lowest value was observed for SH sample (2) which was 0.41%. Statistical analysis showed that treatment TA had a significant effect (p= 0.13) on fiber content of wheat flour. The average values for fiber contents were 0.53, 0.52, 0.49 and 0.55% respectively. The results obtained from the study are in close conformity to the results of Ahmad et al. [23] who reported a range of 0.40% - 0.60% crude fiber content in commercial wheat flour. The crude fiber most likely from the bran of the whole-wheat flour and the hull of soy beans, represents variable fraction of dietary fiber and includes mostly the lignin, cellulose and hemicelluloses components [26, 27]. According to well-documented studies, it is now accepted that dietary fiber plays a significant

role in the prevention of several diseases such as; cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer and diabetes [28, 21].

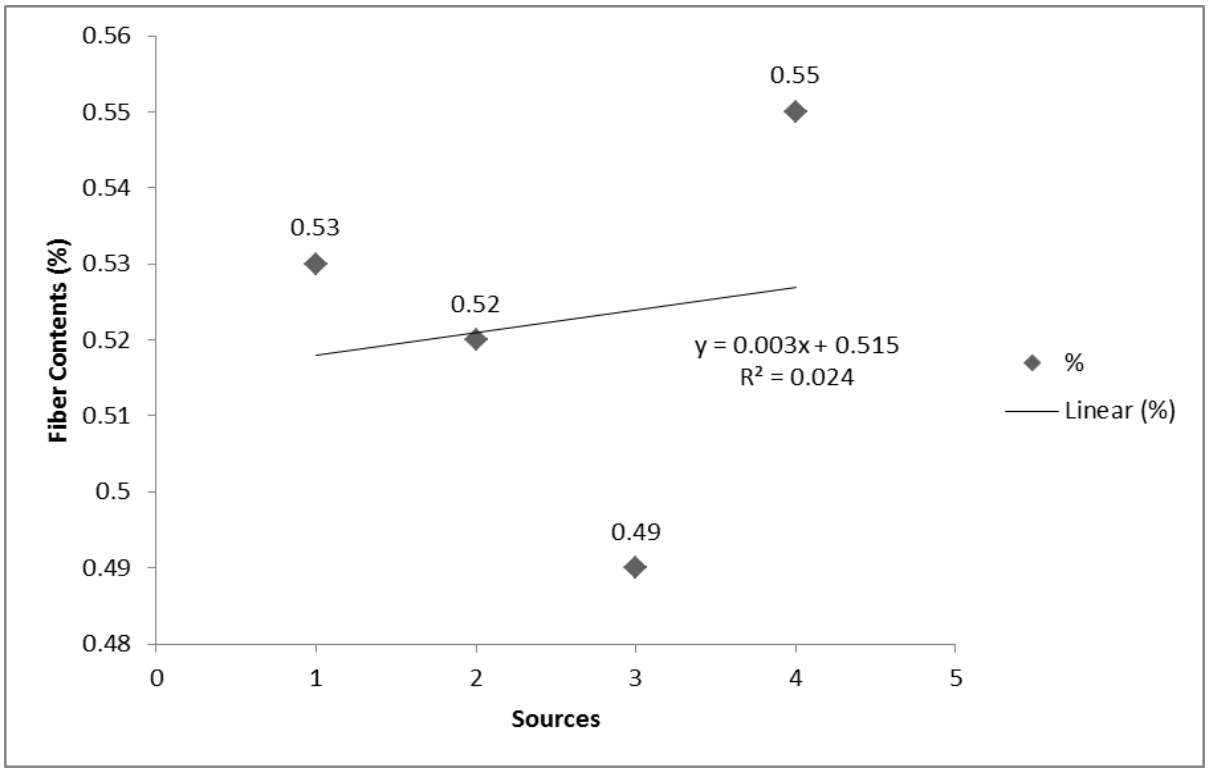


Fig. 4. Fiber content (%) in wheat flour samples collected from different sources, where 1 (FM), 2 (SH), 3(HO) and 4 (TA).

Protein Content

Data collected for protein content of wheat flour samples ranges between 9.06-13.89%. Highest value was observed for SH sample (6) i.e. 13.89 and lowest value was observed for SH sample (8) which was 9.06%. The average values for protein contents were 11.48, 11.62, 10.78 and 11.90% respectively. Statistical analysis showed that there was no significant difference (p= 0.39) among the means of different treatments. The results obtained from the study are similar to the earlier published results and they reported a range of 10.32% - 11.58% protein content in commercial wheat flour [24].

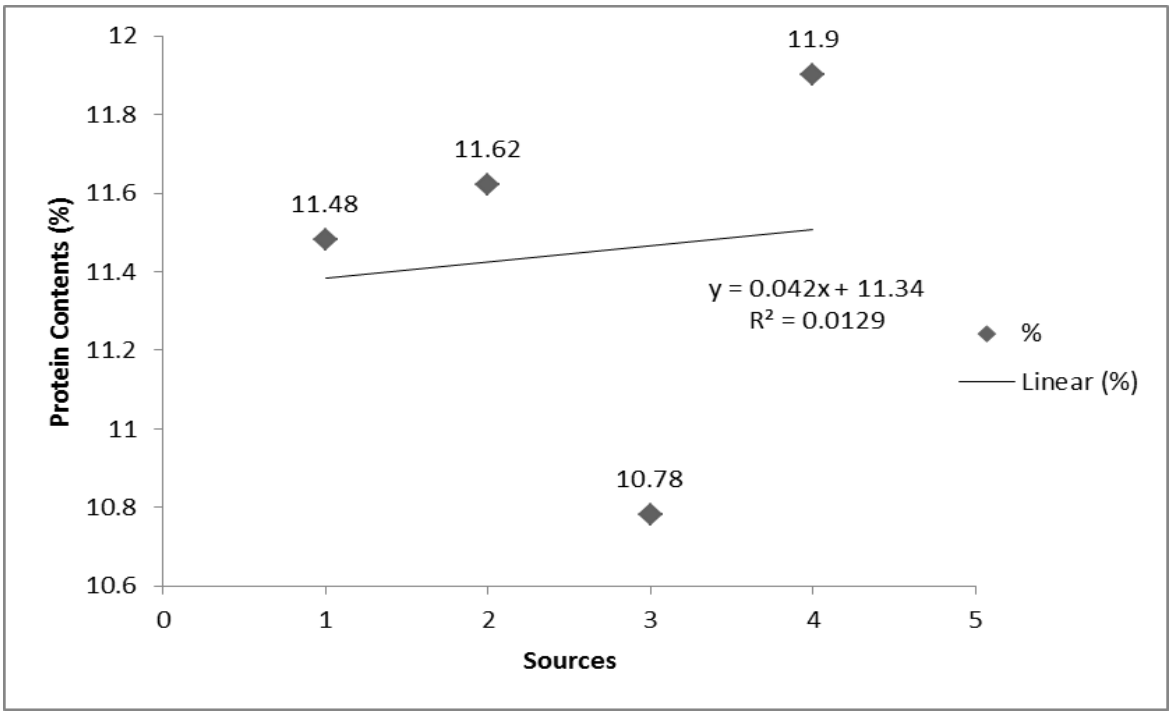


Fig. 5. Protein content (%) in wheat flour samples collected from different sources, where 1 (FM), 2 (SH), 3(HO) and 4 (TA).

Nitrogen Free Extract (NFE)

Data collected for nitrogen free extract (NFE) ranges between 70.31-80.98 %. The highest value was observed for HO sample (5) which was 80.98% while the lowest value was observed for SH sample (4) as 70.31%. The average values were 76.85, 76.13, 77.38 and 77.70% for FM, SH, HO, and TA respectively (Fig. 6). Statistical analysis showed that there was no significant difference (p=0.57) between the means of different treatments. The results obtained from the study are similar to the earlier published results and they reported a range of 74.62% - 77.74% NFE content in commercial wheat flour [24].

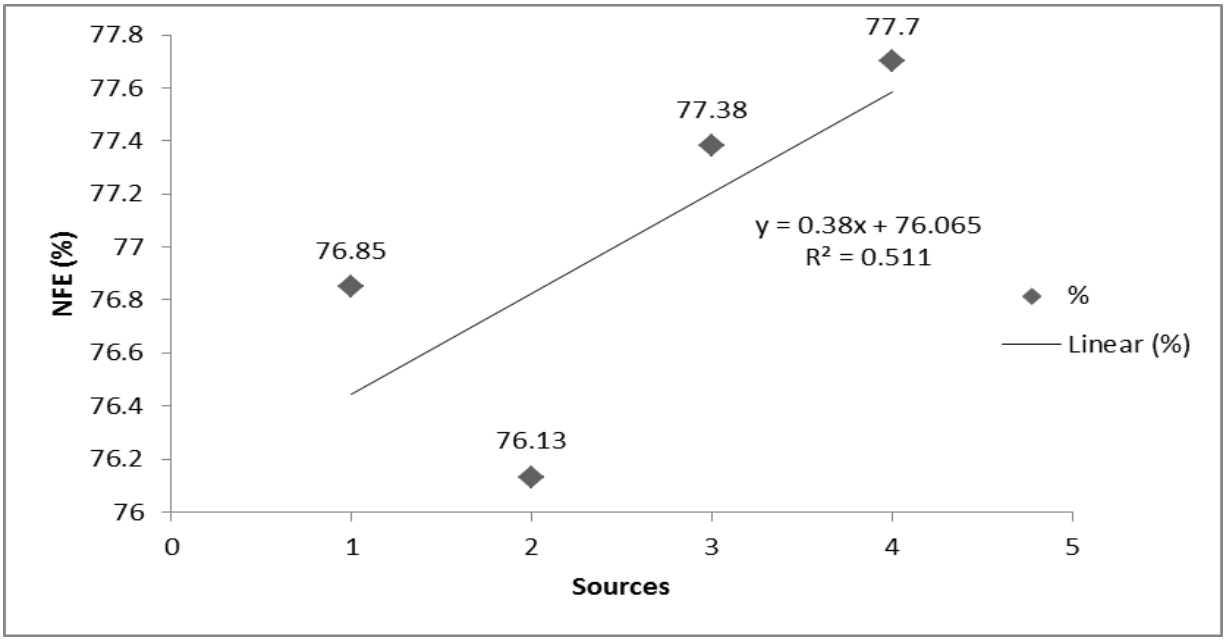
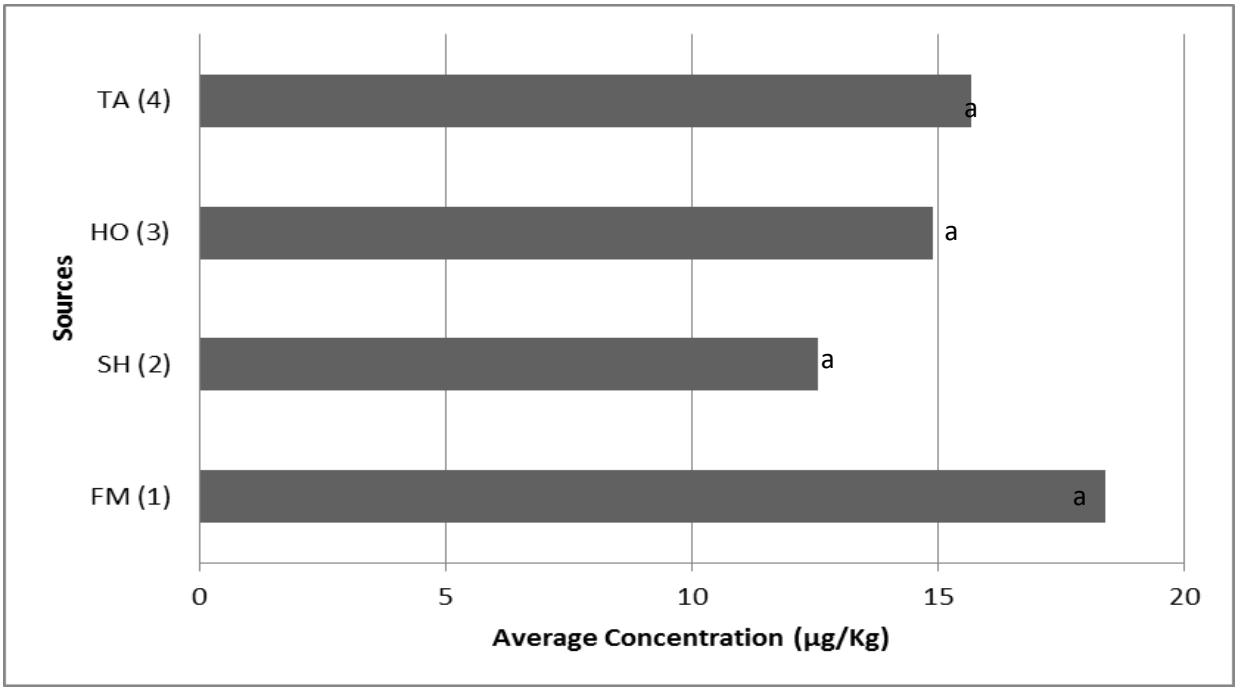


Fig. 6. NFE content (%) in wheat flour samples collected from different sources; where 1 (FM), 2 (SH), 3(HO) and 4 (TA).

Afflation Content of Wheat Flour

Aflatoxin level measured ranges between 00.00 – 34.81 µg/kg. Highest values was observed in samples (9) collected from houses (HO) i.e. 34.81 µg/kg while the lowest value was observed in HO sample (1) and SH sample (5, 10) as 00.00 µg/kg but the average values were 18.40, 12.55, 14.90, 15.67 µg/kg for FM, SH, HO and TA respectively (Fig-7). Statistical analysis showed that there was no significant difference (p= 0.44) among the means of various sources. Results obtained from aflatoxins analysis are similar to the results as that obtained by Makuan et al. [29]. They reported that wheat samples contained 16.2-274µg/kg aflatoxins. Fungal growth and mycotoxin contamination are dependent on climate and storage conditions and therefore vary with locations, with hot and humid climate, poor storage conditions and poor agricultural practices exacerbating fungal and mycotoxin contents in foods and feedstuffs [30](Ominski et al., 1994). Aflatoxin B1 (AFB1)concentrations in the beans (59.29 ± 14.85 ug/kg) and wheat (85.66 ± 16.19 ug/kg) analyzed were above the National Agency for Food and Drug Administration and Control (NAFDAC) and European Union (5 ug/kg) tolerance level for aflatoxin in grains for human consumption. The chronic consumption of these crops with unsafe levels of AFB1, is immunosuppressive, nephrotoxic and hepatocarcinogenic, has grievous public health implications which calls for control and regulation of mycotoxins in the country [31, 32,

33]. The desired control of mycotoxins can be achieved by reducing fungal contamination of crops by rapid drying and correct storage of the harvested crops using effective anti-mould preservatives. Properly designed, mycoflora and mycotoxin surveys and monitoring programmers can reduce the fungal and mycotoxins in our foods. It is high time Nigeria enforced the legislation against mycotoxins.



Mean followed by the same letters are not significantly different at ($p < 0.05$)

Fig. 7. Aflatoxins level (µg/Kg) in wheat flour samples collected from different sources.

CONCLUSION

Wheat flour samples collected from the local areas of Peshawar city were investigated for physico-chemical analysis and aflatoxin content. Proximate analysis of the samples was carried out in order to determine the correlation between the wheat flour compositions with aflatoxins. Results showed a positive correlation among ash, fiber, moisture content and protein to aflatoxins, while the correlation was negative among fat and nitrogen free extract to aflatoxins. Proximate analysis of wheat flour samples showed moisture content from 7.02% -13.88%, ash 0.51% - 0.83%, fat 0.91% - 1.6%, fiber 0.41% - 0.65%, protein 9.06% - 13.89% and NFE 70.31% - 80.98% respectively. Analysis showed that samples collected from HO were heavily

contaminated with aflatoxins i.e. 34.81µg/kg (average 14.90) followed by 30.54µg/kg (average 18.40), 27.31µg/kg (average 12.55) and 26.65µg/kg (average 15.67) in FM, SH and TA respectively, which indicated the level of aflatoxins beyond the safe limit prescribed by FDA (20µg/kg). The average aflatoxin content was 15.38µg/kg. It is concluded that storage of wheat with high moisture content had most probable risk of contamination through toxins so that the grains must be stored at lowest safe moisture level and in dry non-humid conditions.

DECLARATION OF INTEREST

Authors have no any conflict of interest.

REFRENCES

1. Hepper, F.N. (1956). Kew Bulletin 10: 113-34.
2. Hussain T, Abbas S, Khan MA, Scrimshaw NS. (2004). Lysine fortification of wheat flour improves selected indices of the nutritional status of predominantly cereal-eating families in Pakistan. Food Nutr. Bull., 25(2): 114-122.
3. GOP, 2008. Pakistan economic survey 2007-08, finance division, economic adviser wing, Government of Pakistan. Islamabad.
4. Aslam M, Gillani AH, Qazi AR. (1982). Some dimensions of rural food poverty with special emphasis on nutritional status and its improvement Final (Report German Agro Action Project. University of Agricultural Faisalabad, 79.
5. Anjum FM, Walker CE. (1991). A review on the significance of starch and protein to wheat kernel hardness. Journal of Science. Food Agricultural. 56: 1-13.
6. Bodroza-Solarov MJ, Mastilovic N, Mladenov, Vujacic V. (2006). Ecological stability of bread crumb quality number in wheat cultivars. Zito-hleb. 33**:**19-23.
7. Saric M, Torbica A, Zivancev D, Masti-lovic J, Menkovska M. (2005). Vredno-vanje tehnoloskog kvaliteta domacih sorti psenice kao sirovine za pekarsku industriju –

Evaluation of technological quality of do-mestic wheat varieties for the purposes of baking quality. Z*ito-hleb,* 32 (3): 71-83.

8. Ramesh J, Sarathchandra G, Sureshkumar V. (2013). Survey of market samples of food grains and grain flour for Aflatoxin B_1 contamination. Int. J. Curr. Microbiol. App. Sci. 2(5): 184-188.

9. Aidoo KE, Mohamed SM, Candlish AA, Tester RF, Elgerbi AM. (2011). Occurrence of Fungi and Mycotoxins in Some Commercial Baby Foods in North Africa. Food and Nutrition Sciences. 2: 751-758.

10. Bhat RV, Vasanthi S. (2003). Food Safety in Food Security and Food Trade: Mycotoxin Food Safety Risk in Developing Countries. Washington D.C. International Food Policy Research Institute, (Brief 3).

11. Ghasemi-Kebria F, Joshaghani H, Taheri NS, Semnani S, Aarabi M, Salamat F, Roshandel G. (2013). Aflatoxin contamination of wheat flour and the risk of esophageal cancer in a high risk area in Iran. Cancer Epidemiol. 37(3):290-293.

12. Bainton SJ, Coker RD, Jones BD, Morley EM, Nagler MJ, Turner RL. (1980). Mycotoxin training manual; Tropical product institute. London. 1-176.

13. AACC. (2000). Approved methods of American Association of Cereal Chemists. The American Association of Cereal Chemists, St. Pauls Minnessota, USA.

14. Khalil IA, and Saleemullah. (2004). Nitrogen Free Extract. Text book of Chemistry One Bio-Analytical Chemistry. National Book Foundation Islamabad. Pak. 39-40.

15. Abdullah N, Nawawi A, Othman I. (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch based foods. Mycopathalogia. 143: 53-58.

16. Ferreira I, Mendes E, Oliveria M. (2004). Quantification of Aflatoxins B1, B2, G1, and G2 in Pepper by HPLC/Fluorescence. J. liquid Chromatog. 27 (2): 325-334.

17. Steel, R.G.D., J.H. Torrie and D. Dickey.1997. Principles and Procedure of Statistics. A Biometrical Approach 3rd Ed. McGraw Hill BookCo. Inc., New York. pp. 352-358.

18. Joel N, Abdulraheem LO, Zakari UM. (2011). Evaluation of the nutritional and sensory quality of functional breads produced from whole wheat and soya bean flour blends. African J. of Food Science. 5(8): 466 – 472.

19. Riaz A, Wahab S, Hashmi MS, Shah AS. (2007). The influence of mungbean supplementation on the nutritive value of whole wheat flour bread. Sarhad J. Agric. 23(3): 737-742.

20. Akhtar S, Anjum F, Rehman S, Sheikh M, Farzena K (2008). Effect of fortification on the physico-chemical and microbiological stability of whole wheat flour. Food Chem., 112:156-163.

21. Maneju, H., Udobi, C. E., Ndife, J. 2011. Effect of added brewers dry grain on the physicochemical, microbial and sensory quality of wheat bread. Am. J. Food Nutr. 39-43.

22. Elleuch M, Bedigian D, Roiseux O, Besbes S, Blecker C, Attia H (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: Rev. Food Chem., 124: 411-421.

23. Ezeama CF (2007).Food Microbiology: Fundamentals and Applications. Natural Prints Ltd. Lagos.

24. Ahmad I, Ahmad N, Kausar T, Ashraf M. (2005). Effect of maltogenic amylase on the shelf life of bread. Pak. J. Food Sci. 15(3-4): 15-19.

25. Kashlan NB, Srivastava VP, Mohanna NA, Motawa YK, Mameesh, M.S. (1991). The proximate and elemental composition of wheat flour and major types of bread consumed in Kuwait. J. Food Chem. 39 (2):205-210.

26. Mannay S, Shadaksharaswany CM (2005). Foods: Facts and Principles. (2nd ed.). New Age International Ltd. Publishers. New Delhi, India.

27. Islam T, Chowdhury A, Islam M, Islam S (2007). Standardization of Bread Preparation from Soy Flour. Int. J. Sustain. Crop Prod., 2(6):15-20.

28. Slavin JL (2005). Dietary fiber and body weight. Nutr., 21: 411-418.

29. Makun HA, Anjorin ST, Moronfoye B, Adejo FO, Afolabi OA, Fagbayibo G, Balogun BO, Surajudeen AA. (2009). Fungal and aflatoxin contamination of some human food commodities in Nigeria. African J. of food science. 4(4): 127-135.

30. Ominski K, Marquardi RR, Sinha RN, Abramson D. (1994).Ecological aspects of growth and mycotoxin production by storage fungi. In: Miler JD and Trenholm HL (1994). Mycotoxins in grains: Compounds other than aflatoxins. Eagan Press, St. Paul Minnesota, USA pp. 287-314.

31. Gbodi TA, Nwude N. (1998). Mycotoxicosis in Domestic Animals. A Rev. Vet. Hum. Toxicol. 30(3): 235-245.

32. Peraica M, Radic B, Lucic A, Pavolic M. (1999). Diseases caused by moulds in humans Bulletin of the World Health Organization. Bulletin Rev. Vet. Hum. Toxicol. 30(3): 235-245.

33. Peraica M, Radic B, Lucic A, Pavolic M. (1999). Toxic effects of mycotoxins in humans. Bulletin of the World Health Organization. 7: 754-766.