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## Target-Oriented Risk Assessment of AFB<sub>1</sub> in Buckwheat Consumed in Armenia

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### ABSTRACT

Cereals, including buckwheat are consumed as an essential source of energy, minerals, fiber, and vitamins. However, these products are susceptible to aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) infection from various fungi. Considering the wide consumption of buckwheat in Armenia, this study aims to assess the dietary exposure of AFB<sub>1</sub> to Armenian adult population. 24-hour recall method was used to evaluate buckwheat consumption. While AFB<sub>1</sub> contents do not exceed the maximum residue levels, dietary exposure estimates exceed the toxicological reference value for females residing in Tavush region, as well as for consumers of cluster 2 (buckwheat consumption of 64.59 g/day). Hence, the consumption of buckwheat alone has the potential to cause adverse health effects.

### Introduction

Throughout the world's healthy food market, buckwheat grains and their products are considered to have a high nutritional value and low inputs for cultivation (Kerene, et al., 2018, Christa and Soral-Śmietana, 2008). However, many crops, including buckwheat are of concern for mycotoxin contamination, especially in the light of recent rapid changes in the weather conditions which affect all the aspects of food security (Leggieri, et al., 2012, Kerene, et al., 2018). Mycotoxins are produced by fungi which colonize many crops and adapt to a wide range of environmental conditions (Khodaei, et al., 2021, Xiong, et al., 2021). In the food and feed industry, the most concerning mycotoxins are produced by *Aspergillus*, *Fusarium* and *Penicillium* (Das, et al., 2021).

Till now, 400 types of mycotoxins have been identified, out of which a few are dangerous to humans and animals, including aflatoxins (AFs), ochratoxins, deoxynivalenol, fumonisins, and zearalenone (Khodaei, et al., 2021). A key point of interest in relation to buckwheat are the AFs which are potent carcinogens that exist in five ways: AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> and M<sub>1</sub> (EFSA, 2013, Mahapatra, 2021). The International Agency for Research on Cancer (IARC) has classified AFB<sub>1</sub> as a Group 1A carcinogen (carcinogenic to humans) (IARC, 1993). About 4.5 billion people worldwide are chronically exposed to low aflatoxin levels due to the daily consumption of products contaminated with it (Fan, et al., 2021, Pickova, et al., 2021). At high concentrations, the consumption can lead to serious health problems, including damage to the liver and other

organs, causing liver cancer, and death (WHO, 2020).

Considering the adverse health effects of mycotoxins on humans and animals, many countries, including the European Union (EU), set up standards for maximum levels of total aflatoxins in food products intended for both human and animal consumption. The EU established the maximum acceptable limits for AFs in nuts, dried fruits, cereals and cereal products, spices, and milk (Kortei, et al., 2021, Palumbo, et al., 2020).

To protect human health, it is essential to assess the presence of mycotoxins in cereals, including buckwheat. Previously, the Center for Ecological Noosphere Studies of Armenia (CENS) carried out investigations regarding concentrations of mycotoxins, including AFB<sub>1</sub> in cereals and cereal-based products and health risk assessment through cereal consumption in Yerevan, Armenia. The results of these studies indicate that contamination level of AFB<sub>1</sub> in cereal crops such as rice, buckwheat, maize and wheat sold in markets of the city of Yerevan range from 1.06 to 3.11 µg/kg, though the concentrations do not exceed the acceptable level (Pipoyan, et al., 2016). Meanwhile, the daily intake of AFB<sub>1</sub> via total consumption of cereal crops, in particular rice, buckwheat, maize and wheat, consumed by Yerevan population exceeds the toxicological reference value for AFB<sub>1</sub>, indicating a potential health risk (Pipoyan, et al., 2017). However, individual consumption of the studied cereals didn't exceed the reference value. It is worth mentioning, that the previous investigations were carried out for the whole studied population, without taking into consideration different groups of population. Taking into consideration the absence of similar studies in the whole territory of Armenia, this is the first-ever attempt to carry out a target-oriented dietary exposure assessment of AFB<sub>1</sub> in buckwheat both in the capital of Armenia, as well as in other regions.

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## Materials and methods

### *Data collection and statistical analysis*

The data on AFB<sub>1</sub>'s concentrations in buckwheat samples was obtained from previous studies (Pipoyan, et al., 2016, Pipoyan, et al., 2017). Detailed information regarding sample collection and analysis is presented there. The ELISA method was used to determine AFB<sub>1</sub> concentrations and the limit of detection (LOD) of the method has been estimated to be 0.001 mg/kg.

To obtain buckwheat consumption data, a 24-hour dietary

recall (24HR) was developed and used for conducting surveys. The survey was conducted in the frame of the research project on “Strengthening scientific and methodological capacity for assessing food security and nutrients” (20TTTCG-4A001).

The 24HR study was conducted in 2021 and included all the regions of Armenia, as well as all the districts of Yerevan to ensure national representativeness. One thousand and four hundred people (18–80 years old and over) residing in Armenia took part in the survey. The 24HR survey was paper-based and interviewer-administered. The average daily consumption of buckwheat was calculated considering the consumption frequency and the portion size. All the statistical analyses were conducted using SPSS software (version 22). Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check for normality. To get a normal distribution of consumption values, the K-means cluster analysis method was applied. Two homogeneous cluster groups were revealed.

### *Exposure assessment*

By combining AFB<sub>1</sub>'s concentration data with consumption data, estimated daily intake (EDI) was calculated:

$$EDI = \frac{C \times IR}{BW},$$

where *EDI* is the exposure to AFB<sub>1</sub> (ng/kg bw/day), *C* is the mean concentration of contaminant (µg/kg), *IR* is the rate of ingestion of food (g/day), *BW* is the body weight (kg) (mean body weights for males and females in studied regions were 77 and 66 kg, respectively). Data regarding weight was self-reported through 24HR.

## Results and discussions

### *Content of AFB<sub>1</sub> in buckwheat*

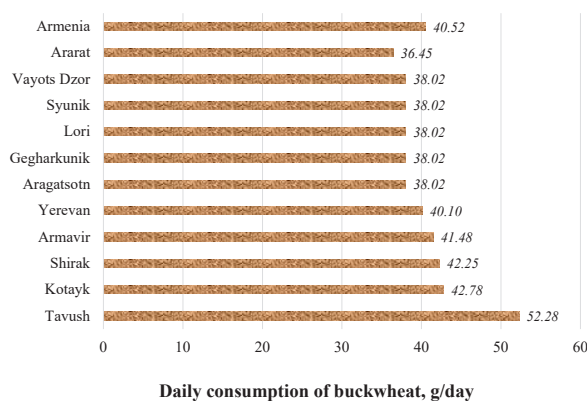
The contents of AFB<sub>1</sub> in buckwheat samples ranged from 1.06 µg/kg to 1.89 µg/kg, with a mean of 1.475 µg/kg. According to the Customs Union's Technical Regulation on Food Safety (TR CU 021/2011), the content of AFB<sub>1</sub> in cereals do not exceed 5 µg/kg. In contrast, the European Commission Regulation No. 1881/2006 has set a maximum residue level (MRL) of 2 µg/kg of AFB<sub>1</sub> in all cereals and cereal products (buckwheat is included in cereals) (EC, 2006). Although the detected AFB<sub>1</sub> contents do not exceed the MRL, they make up 53 %-73 % of it.

The AFB<sub>1</sub> concentrations of the current study are comparable with the values estimated in other countries. A study towards risk assessment for aflatoxin B<sub>1</sub> in Japan

indicated that buckwheat is among the major contributors of AFB<sub>1</sub> intake. The contents of AFB<sub>1</sub> in buckwheat flour ranged from 0.04 – 0.08 µg/kg (Sugita-Konishi, et al., 2010). These values are substantially lower than the ones of the current study (1.06 – 1.89 µg/kg).

### Buckwheat consumption

The average buckwheat consumption for the studied population is 40.52 g/day; 54 % of consumers are female (with an average buckwheat consumption of 40.5 g/day) and 46 % are male (with an average consumption of 40.4 g/day). A mean consumption data for Armenia, as well as a separate data for all 11 regions is represented in Figure 1. The amounts ranged from 36.45 g/day in Ararat region to a maximum of 52.28 g/day in Tavush region.



**Figure 1.** Buckwheat consumption (g/day) across Armenia (composed by the authors).

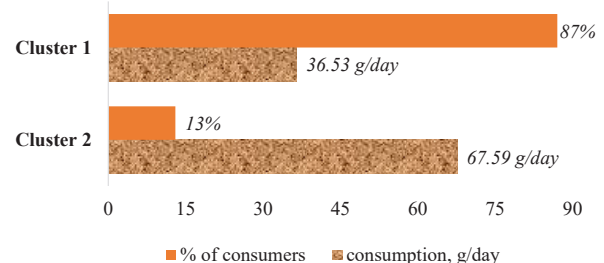
According to the cluster analysis 2 clusters were revealed. The consumption amounts of buckwheat for two different clusters are presented in Figure 2. For the first and second clusters, the consumption amounts are equal to 36.53 g/day and 67.59 g/day, respectively. While the consumption amount increases from the first to the second cluster, the amount of people in each cluster decreases. While cluster 1 includes most consumers (87 %), cluster 2 includes only a small portion of it (13 %).

### EDI of AFB<sub>1</sub>

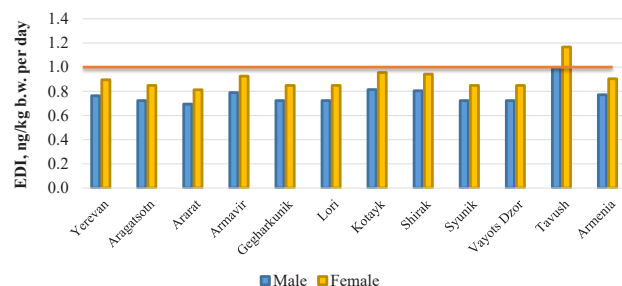
The EDI values have been calculated for the whole country, each region, as well as for each cluster and gender. The amounts for cluster 1 and 2 are equal to 0.753 and

1.394 ng/kg bw/day, respectively. The average EDI obtained for the whole studied population is equal to 0.835 ng/kg bw/day. The average EDI obtained for the Yerevan population is equal to 0.827 ng/kg bw/day. Overall, the exposure to AFB<sub>1</sub> contamination is higher among females (0.771 ng/kg bw/day) than males (0.771 and 0.904 ng/kg bw/day). The highest daily intake of AFB<sub>1</sub> has been estimated in Tavush region and the lowest in Ararat region (Figure 3).

Considering the toxicological reference value of 1 ng/kg bw/day for AFB<sub>1</sub> defined by Scientific Committee on Food (SCF) and Expert Committee on Food Additives (JECFA), the EDI values obtained for females residing in Tavush region, as well as for consumers of cluster 2 exceed the threshold in 1.07 and 1.39 times, respectively (Leblanc, et al., 2005). It is of particular importance to state that the rest of the EDI values make up from 75 % to 88 % of the toxicological reference value. Therefore, there is a very high risk that the intake of AFB<sub>1</sub> through buckwheat alone can pose potential health risks for humans, particularly for the diabetic consumers who have the highest intake of buckwheat.



**Figure 2.** Buckwheat consumption (g/day) based on clusters (composed by the authors).



**Figure 3.** Estimated daily intake of AFB<sub>1</sub> (ng/kg bw per day) (composed by the authors).

## Conclusion

The obtained results indicate, that although in the buckwheat samples contents of AFB<sub>1</sub> do not exceed the MRL, they make up 53 % – 73 % of it. Meanwhile, the consumption of buckwheat leads to EDI values exceeding the toxicological reference value in case of females residing in Tavush region, as well as for consumers of cluster 2 in 1.07 and 1.39 times, respectively.

Due to fact that buckwheat can contain other toxic substances, including heavy metals, constant monitoring and exposure assessment are important for protecting consumer health. It has been shown that heavy metals such as nickel, arsenic, lead, chromium, mercury, and cadmium share the main target organ, liver, with AFs, and it is therefore important to research their interactions (Renu, et al., 2021). This is particularly relevant for Armenia, since many risk assessment studies conducted in various regions of Armenia indicated the adverse effects of the above stated heavy metals for human health through the consumption of various food items (Pipoyan, et al., 2019, 2020, 2022).

Hence, in future investigations, it is recommended to assess the risk of other substances in buckwheat, as well as in other commonly consumed cereal-based products to better understand the overall risk of cereal products consumed in Armenia.

## References

- Christa, K., and Soral-Śmietana, M. (2008). Buckwheat Grains and Buckwheat Products - Nutritional Fagopyrum esculentum and Selected Buckwheat Product. *Czech Journal of Food Science* 26, - pp. 153-162. <https://doi.org/10.17221/1602-cjfs>.
- Das, A., Mandal, S., Nag, S., and Mondal, B. (2021). Management of Storage Pathogens of Cereal Grains: A Review.
- EC, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs / Official Journal of the European Union L364, - pp. 5-24.
- EFSA, 2013. Aflatoxins (Sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>) in Cereals and Cereal-Derived Food Products. EFSA Supporting Publications 10: 406E. <https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2013.EN-406>.
- Fan, T., Xie, Y., and Ma, W. (2021). Research Progress on the Protection and Detoxification of Phytochemicals against Aflatoxin B<sub>1</sub>- Induced Liver Toxicity. *Toxicol*, 195, - pp. 58-68. [https://doi.org/10.1016/0003-2670\(94\)80328-5](https://doi.org/10.1016/0003-2670(94)80328-5).
- IARC, 1993. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. In *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*; World Health Organization, Ed.; IARC Press: Lyon, France. Volume 56, - pp. 445-466.
- Kerlene, I., Mankeviciene, A., and Cesnuleviciene, R. (2018). Risk Factors for Mycotoxin Contamination of Buckwheat Grain and its Products. *World Mycotoxin Journal*, 11(4), -pp. 519-529. <https://doi.org/10.3920/wmj2018.2299>.
- Khodaei, D., Javanmardi, F., and Khaneghah, A. M. (2021). The Global Overview of the Occurrence of Mycotoxins in Cereals: a Three-Year Survey. *Current Opinion in Food Science*, 39, - pp. 36-42. <https://doi.org/10.1016/j.cofs.2020.12.012>.
- Kortei, N.K., Annan, T., Kyei-Baffour, V., Essuman, E.K., Okyere, H., and Tettey, C.O. (2021). Exposure and Risk Characterizations of Ochratoxins A and Aflatoxins through Maize (*Zea mays*) Consumed in Different Agro-Ecological Zones of Ghana. *Scientific Reports*, 11(1), - pp. 1-19. <https://doi.org/10.1038/s41598-021-02822-x>.
- Leblanc, J.C, Tard, A., Volatier, J.L., Verger, P. (2005). Estimated Dietary Exposure to Principal Food Mycotoxins from the First French Total Diet Study, *Food Additives and Contaminants*, 22:(7):652-672. <https://doi.org/10.1080/02652030500159938>.
- Leggieri, M.C., Toscano, P., and Battilani, P. (2021). Predicted Aflatoxin B<sub>1</sub> Increase in Europe due to Climate Change: Actions and Reactions at Global Level. *Toxins*, 13(4), - 292 p. <https://doi.org/10.3390/toxins13040292>.
- Mahapatra, S.S. (2021). Antioxidants as Modulators of Plant Defense against Soilborne Fungal Pathogens upon Microbial Interaction. In *Antioxidants in Plant-Microbe Interaction*. Springer, Singapore, - pp. 305-314. [https://doi.org/10.1007/978-981-16-1350-0\\_14](https://doi.org/10.1007/978-981-16-1350-0_14).
- Palumbo, R., Crisci, A., Venâncio, A., Cortiñas Abrahantes, J., Dorne, J.L., Battilani, P., and Toscano, P. (2020). Occurrence and Co-Occurrence of Mycotoxins in Cereal-Based Feed and Food. *Microorganisms*, 8(1), - 74 p. <https://doi.org/10.3390/microorganisms8010074>.

14. Pickova, D., Ostry, V., Toman, J., and Malir, F. (2021). Aflatoxins: History, Significant Milestones, Recent Data on their Toxicity and Ways to Mitigation. *Toxins*, 13(6), - 399 p. <https://doi.org/10.3390/toxins13060399>.
15. Pipoyan, D., Hovhannisyan, A., Stepanyan, S., Ghrejyan, E. (2017). Dietary Exposure of Aflatoxin B<sub>1</sub> via Consumption of Cereals in Yerevan. *Bulletin of National Agrarian University of Armenia*. - Vol. 4, - pp. 143-146.
16. Pipoyan, D., Stepanyan, S., Beglaryan, M., Dorne, J.L. (2022). Risk Assessment of Uptake of Trace Elements through Consumption of Cereals: a Pilot Study in Yerevan, Armenia. *Journal of Environmental Health Science and Engineering*, - pp. 1-10.
17. Pipoyan, D., Stepanyan, S., Beglaryan, M., Stepanyan, S., Asmaryan, S., Hovsepyan, A., and Merendino, N. (2020). Carcinogenic and Non-Carcinogenic Risk Assessment of Trace Elements and POPs in Honey from Shirak and Syunik Regions of Armenia. *Chemosphere*, 239, 124809.
18. Pipoyan, D., Stepanyan, S., Stepanyan, S., Beglaryan, M., and Merendino, N. (2019). Health Risk Assessment of Potentially Toxic Trace Elements in Vegetables Grown under the Impact of Kajaran Mining Complex. *Biological Trace Element Research*, 192(2), - pp. 336-344.
19. Pipoyan, D.A., Galoyan, G.M., Hovhannisyan, A.S. (2016). Assessment of Aflatoxin B<sub>1</sub> Contamination Level in Cereals Sold in Yerevan, *Agroscience Scientific Journal*, 3-4, - pp. 101-103.
20. Renu, K., Chakraborty, R., Myakala, H., Koti, R., Famurewa, A.C., Madhyastha, H., Vellingiri, B., George, A., Valsala Gopalakrishnan, A. (2021). Molecular Mechanism of Heavy Metals (Lead, Chromium, Arsenic, Mercury, Nickel and Cadmium) Induced Hepatotoxicity. *Chemosphere*. 271:129735. [https://doi: 10.1016/j.Chemosphere.129735](https://doi:10.1016/j.Chemosphere.129735).
21. Sugita-Konishi, Y., Sato, T., Saito, S., Nakajima, M., Tabata, S., Tanaka, T., Norizuki, H., Itoh, Y., Kai, S., Sugiyama, K., Kamata, Y., Yoshiike, N. and Kumagai, S. (2010). Exposure to Aflatoxins in Japan: Risk Assessment for Aflatoxin B<sub>1</sub>, Food Additives and Contaminants: Part A, 27:3, 365-372, <https://doi:10.1080/19440040903317497>.
22. TR CU 021/2011. Technical Regulations of the Customs Union “On Food Safety”. <https://docs.cntd.ru/document/902320560> (accessed on 05.03.2022).
23. WHO, 2020. Aflatoxins. Available at: <https://www.mdpi.com/2072-6651/14/5/307> (accessed on 07.03.2022).
24. Xiong, Z., Wang, Q., Xie, Y., Li, N., Yun, W., and Yang, L. (2021). Simultaneous Detection of Aflatoxin B<sub>1</sub> and Ochratoxin A in Food Samples by Dual DNA Tweezers Nanomachine. *Food Chemistry*, 338, 128122.

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