SCIENTIFIC JOURNAL

ISSN 2466-2852

# meat technology

61 Vol. 01 No. 2020 Belgrade

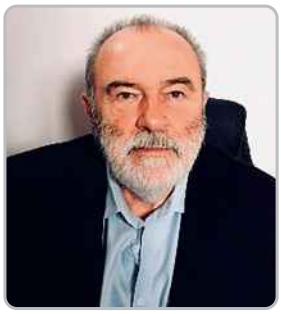
Founder and Publisher
Institute of Meat Hygiene
and Technology

"Meat Technology" is the scientific journal that publishes results of basic and applied research in the field of biotechnical sciences i.e. the following subcategories: veterinary sciences, food engineering and biotechnology.

Journal "Meat Technology" is abstracted in FSTA (Food Science and Technology Abstract). Full text is available in CABI Database, EBSCO publishing, DOAJ, AGRIS, www.ifocus.my Database and www.inmes.rs. "Meat Technology" is an open access journal. 

# Editorial board of Scientific Journal "Meat Technology"

# *On the occasion of 60 years of the journal Meat Technology*



*In only ten years after the end of the Second World War, Yugoslavia made significant progress in all industrial areas. This progress was particularly pronounced in the meat industry thanks to changes in livestock production. Genetic selection in cattle and pig production favoured high-yielding meat breeds, which resulted in higher meat production. Previously, the slaughter industry did not have the capacity or equipment to process the existing supply of livestock for slaughter. It is well known that the export of live animals for slaughter is economically far less profitable than the export of meat, and especially meat products. This initiated the need to build new slaughterhouses and processing facilities of industrial type, following the example of those in the USSR and the USA, and where there were possibilities, to increase the capacity of already existing slaughter and meat processing facilities. At the same time, special importance was given to increasing the meat processing capacities, which would ensure the supply of the domestic market, but also increase the volume of exports of meat products. In order to realize their intentions and wishes as successfully as possible, the already existing companies, as well as those under construction, founded the Association of the Meat Industry. The idea of establishing a scientific institution, which would help them in the realization of the planned activities, was clearly expressed in the plans of this Association. The idea was realized as early as 1955, when the Yugoslav Institute of Meat Technology was founded, which, as expected, had numerous and complex tasks ahead of it. The staff of the Institute, although initially small in number of employees and without sufficient equipment, was not afraid of difficulties or challenges. The Institute had the support of experts in the meat industry, and conviction that with joint work and the desire for constant improvement, the meat industry will be successful. The need for constant mutual contacts and cooperation between the meat industry and the Institute resulted, three years later, in 1958, in the First Conference of the Meat Industry. The main purpose of the conference was for science to serve the meat industry, and the meat industry to be a source of new scientific thoughts, ideas and challenges. The Institute and the meat industry aspired, as always, to higher, better and more efficient interconnection. That new, "golden", perhaps the strongest link, between the science and industry was the journal. Thus, in August 1960, the first issue of the journal Meat Technology was published by the Institute. The first editor was Prof. Isidor Savić, PhD, expert and scientific head of the Institute. The journal has become a source of new knowledge and information. The authors of original research papers, reviews, short communications, reviews from the meat industry and foreign literature are associates of scientific institutions, faculties, experts from the meat industry, government agencies, etc. The journal was readily accepted by readers of various educational profiles. From the very beginning, it has been broadly focused on multidisciplinarity. For everyone who deals with meat and what is related to meat, there was and is a place in the journal. For all these years, and there are 60 of them, the journal was, is and we believe that it will remain rich in the diversity of content, which depicts the physiognomy of a progressive, modern and future-oriented meat industry.*

*The current way of informing in science is increasingly focused on new technologies, so the journal Meat Technology in electronic form is available to all interested today, and as it is printed in English, it has a good chance of becoming appreciated in international scientific circles. Today, by entering the keyword meat in Kobson database, one can find seven journals with this word in the title, and only one of them (Meat Science, published since 1977, M21) has an impact factor. The five journals entitled meat are more journals dedicated to the marketing (trade) of meat and equipment. By entering the German word for meat "das Fleisch" in Kobson database, we come to four journals that have this word in the title. Only one of them has an impact factor (Fleischwirtschaft, published since 1920, M23). A significant number of journals in the world publish papers in the field of meat hygiene and technology, but there is no "meat" in their titles. Maybe that is exactly what gives Meat Technology the uniqueness, the recognisability in the world that the journal Meat Technology had in Serbia (Yugoslavia) while it was published in the Serbian language as Tehnologija mesa.*

*Today and in the future, appreciating the contribution of Meat Technology to the meat science and its importance to the meat industry, given the advancement of scientific thought and the steady progress of the meat industry, we must continue to work to improve cooperation between science and meat industry, as single impartible organism which survives, lasts, and thrives in communion. The editors, editorial boards, authors of the journal have always been faced with new challenges which they have met the belief, optimism and confidence in their abilities, and that is the key to the future of Meat Technology.*

*A word from the honorary editor, Prof. Milan Ž. Baltić, PhD*

# *Povodom 60 godina izlaženja časopisa Tehnologija mesa*

*Za samo desetak godina posle završetka Drugog svetskog rata, Jugoslavija je u svim industrijskim oblastima ostvarila značajan napredak. Taj napredak bio je naročito izražen u industriji mesa zahvaljujući promenama u stočarskoj proizvodnji. Genetskom selekcijom u govedarstvu i proizvodnji svinja favorizovane su rase sa većim prinosom mesa, što je rezultiralo i većom proizvodnjom mesa. Dotadašnja klanična industrija nije imala ni te kapacitete ni opremu da prihvati svu ponuđenu stoku za klanje. Dobro je poznato da je izvoz živih životinja za klanje ekonomski daleko manje isplativ od izvoza mesa, a naročito proizvoda od mesa. To je iniciralo potrebu izgradnje novih klaničnih i preradnih kapaciteta industrijskog tipa po uzoru na one u SSSR i SAD, a tamo gde je bilo mogućnosti i povećavanje kapaciteta već postojećih objekata za klanje stoke i preradu mesa. Pritom, poseban značaj dat je povećanju preradnih kapaciteta mesa, koji bi osigurali snadbevanje domaćeg tržišta, ali i povećali obim izvoza proizvoda od mesa. Da bi što uspešnije realizovali svoje namere i želje, već postojeća preduzeća, kao i ona u izgranji, osnovala su Udruženje industrije mesa. U projekcijama ovog Udruženja bila je jasno izražena i ideja o osnivanju naučne institucije, koja bi im pomogla u realizaciji planiranih aktivnosti. Ideja je realizovana već 1955. godine, kada je osnovan Jugoslovenski institut za tehnologiju mesa, pred kojim su, očekivano, bili brojni i složeni zadaci. Kolektiv Instituta, mada u početku kadrovski malobrojan i bez dovoljno opreme, nije se plašio ni poteškoća, ni izazova. Znao je da u industriji mesa, u praksi, ima podršku zaposlenih stručnjaka i da će uz zajednički rad i želju za stalnim unapređenjem industrija mesa biti uspešna. Potreba za stalnim međusobnim kontaktima i saradnjom između industrije mesa i Instituta rezultirala je, već tri godine kasnije, 1958. godine, održavanjem Prvog savetovanja industrije mesa. Osnovna svrha savetovanja bila je da nauka služi praksi, a praksa da bude izvor novih naučnih misli, ideja i izazova. Institut i industrija mesa težili su, kao i uvek, ka višem, boljem i efikasnijem međusobnom povezivanju. Ta nova „zlatna", možda i najjača karika, između nauke i prakse bio je časopis. Tako je 1960. godine avgusta meseca u izdanju Instituta štampan prvi broj časopisa Tehnologija mesa. Prvi urednik bio je prof. dr Isidor Savić, stručni i naučni rukovodilac Instituta. Časopis postaje izvor novih znanja i informacija. Autori originalnih istraživačkih radova, revijalnih prikaza, saopštenja, prikaza iz prakse i strane literature su saradnici naučnih institucija, fakulteta, stručnjaci iz prakse, državnih organa. Časopis Tehnologija mesa je vrlo rado prihvaćen kod čitalaca različitih obrazovnih profila. On je od samih početaka široko okrenut multidisciplinarnosti. Za svakoga ko se bavi mesom i onim što je u vezi sa mesom, u časopisu je bilo i ima mesta. Za sve ove godine, a 60 ih je, časopis je bio, jeste i verujemo da će ostati bogat raznovrsnošću sadržaja, koji oslikava fizionomiju jedne progresivne, savremene i budućnosti okrenute industrije mesa.*

*Ovovremeni način informisanja u nauci sve je više okrenut novim tehnologijama, pa je i časopis Tehnologija mesa u elektronskom obliku danas dostupan svim zainteresovanim, a kako se štampa na engleskom jeziku, ima dobre izglede da postane cenjen i u međunarodnim naučnim krugovima. Danas se unošenjem ključne reči meat u bazu Kobsona može naći sedam časopisa koju naslovu imaju ovu reč, a samo jedan od njih (Meat Science, izlazi od 1977. godine, M21) je sa impakt faktorom. Pet časopisa sa nazivom meat su više magazini posvećeni prometu (trgovini) mesa i opreme. Unošenjem nemačke reči za meso fleisch u Kobsonovu bazu, dolazi se do četiri časopisa koji u naslovu imaju ovu reč. Samo jedan od njih ima impakt faktor (Fleischwirtschaft, izlazi od 1920. godine, M23). Znatan broj časopisa u svetu objavljuje radove iz oblasti higijene i tehnologije mesa, ali u svom naslovu nema ono meat. Možda je to upravo ono što časopisu Meat Technology daje posebnost, prepoznatljivost u svetu kakvu je časopis imao u Srbiji (Jugoslaviji) dok je izlazio na srpskom jeziku kao Tehnologija mesa.*

*Danas i u budućnosti, ceneći doprinos časopisa Meat Technology nauci o mesu i značaj za industriju mesa, moramo, s obzirom na unapređenje naučne misli i stalan napredak industrije mesa, i dalje da radimo na unapređenju saradnje između nauke i prakse, jer je to jedan nedeljiv organizam koji opstaje, traje i živi u samom zajedništvu. Pred urednicima, uređivačkim odborima, autorima radova časopisa, uvek je bilo novih izazova kojima se išlo u susret sa verom, optimizmom i sigurnošću u svoje mogućnosti, a to je i ključ budućnosti časopisa Meat Technology.*

*Reč počasnog urednika, prof. dr Milana Ž. Baltića*

# *The journal Meat Technology (Tehnologija mesa) today*

*Tehnologija mesa (since 2016 Meat Technology), a journal of the meat industry of Yugoslavia, was founded in 1960, five years after the founding of the Yugoslav Institute of Meat Technology (1955) and, since then, has been published continuously to this day. The journal was started by the conclusion of the Steering Committee of the Association of Canning Industry of Yugoslavia, as a body of that Association, and by the conclusion of the Steering Committee of the Institute of Meat Technology of the Federal People's Republic of Yugoslavia, from April 1961, the journal was taken over as a body of the Institute. Today, the founder and publisher of the journal is the Institute of Meat Hygiene and Technology from Belgrade.*

*The journal Meat Technology appears at a time when the meat industry began to gain the reputation of a significant industry in the newly established country, its role was to contribute to establishment of the connection, link between science and meat industry, i.e. to enable the exchange of experiences between researchers from the Institute and experts from the meat industry, to inform on the development and possibilities of the meat industry in other countries and to publish the results achieved, both in the Institute and in other scientific institutions. The journal published original research papers and articles in the field of production and processing of all types of meat important for human consumption, also in the field of production and processing of eggs, animal fats and oils, production and use of additives, packaging materials and equipment used in manufacturing of meat products, as well as in the field of microbiology, chemistry and contamination of meat and meat products with biological residues. The content and importance of the journal and published papers reflected the current state and development of the Institute and the meat industry and other related industries, as well as association with numerous scientific institutions in the country and abroad. Since 1960, the journal has published the results of basic and applied research in the field of biotechnical sciences, i.e. branches: veterinary medicine, food engineering and biotechnology. With a multidisciplinary approach to all scientific and vocational problems, different profiles of scientific researchers and experts have been mobilized with the aim of uniting the potential from several scientific fields and giving a more significant contribution to the development of the meat science.*

*The development of the science, both in the world and in our country, brings legal regulations (laws, regulations and/or acts), which define and regulate the status of the journal. A legal Act on the regulation of international journals, the scientific status of each publication at the state level is recognized within the categorization of journals as stipulated by the law. Subject to the categorization are journals deposited in the Repository of the National Library of Serbia, in paper and electronic form, for permanent storage and meet the requirements of the Act. The Centre for Evaluation in Education and Science (CEON) was established in 2002 as an autonomous research and development agency. The main activity of CEON was the development of bibliometric methods and databases intended for evaluation and, at the same time, promotion of research results generated in Serbia and other countries in Southeast Europe. CEON was the organization that performed all annual analyses of the journals for the needs of the competent ministries. Since 2016, the Ministry of Education, Science and Technological Development has hired the Mathematical Institute of SANU to prepare, maintain and publish a bibliometric report on scientific journals for the needs of categories and ranking of journals in the Republic of Serbia. Serbia. In accordance with that, the categorization of journals of national importance and evaluation of scientific work is still performed today. Papers in national journals can have the following categories: M24 — journal of the international importance verified by a special decision of the scientific committee of the competent Ministry; M51 — leading journal of national importance; M52 — journal of national importance and M53 — scientific journal.*

*From its founding to year 1988, the journal was published once a month, then every two months, then three times a year, and since 2010 it is published twice a year. This was a consequence of the reduced number of manuscripts received by the editorial board of the journal, i.e. the result of the political and economic turmoil in this area. Until 2015, the papers in the journal were published in Serbian language, with abstracts and keywords, tables, graphs and pictures in Serbian and English. In addition, some papers have been published in English, German or Russian. In that case, their Summaries, tables, graphs and pictures were printed in the original language of the paper and in Serbian. In order to become available to the wider scientific public, in 2016 the journal began to be published in English, with abstracts of papers, in addition to English, also in Serbian. Until 2015, the magazine was entitled Tehnologija mesa (ISSN 0494-9846), and since 2016 Meat Technology (ISSN 2466-4812).*

*The journal Meat Technology has maintained a high scientific level, in very complex conditions for the development of research and transfer of research results to the meat industry — production, processing and control of meat, and followed all requirements for the evaluation of scientific work, in accordance with the development of bibliometric methods necessary for ascent on the categorization scale and ranking of journals at the national level. At the beginning of its publication, in accordance with the set goals, the journal achieved the category M53 rank — scientific journal and/or M52 — journal of national importance. From 2009 to 2016, it was mainly the category M51 — the leading journal of national importance, and since 2017 it has continuously been ranked the category M24 — the journal of international importance verified by a special decision of the Main Scientific Committee for Biotechnology and Agriculture of the competent Ministry. It is important to note that the engagement of scientists in writing, publishing and citing papers from Meat Technology in journals on the SCI list (Science Citation Index) will contribute not only to maintaining the status of journal in the achieved category, but also to its rise to a higher, international level.*

*PhD Aurelija Spirić, retired Principal research fellow*

# *Časopis Tehnologija mesa (Meat Tecnology) danas*

*Tehnologija mesa (od 2016. godine Meat Technology), časopis industrije mesa Jugoslavije, osnovan je 1960. godine, pet godina nakon osnivanja Jugoslovenskog instituta za tehnologiju mesa (1955. godine) i, od tada, izlazi neprekidno do današnjeg dana. Časopis je osnovan zaključkom upravnog odbora Udruženja konzervne industrije Jugoslavije, kao organ tog Udruženja, a zaključkom Upravnog odbora Instituta za tehnologiju mesa FNRJ od aprila 1961. godine, časopis je preuzet kao organ Instituta. Danas je osnivač i izdavač časopisa Institut za higijenu i tehnologiju mesa iz Beograda.*

*Časopis Tehnologija mesa se pojavljuje u vreme kada je industrija mesa počela da stiče ugled jedne značajne industrijske grane u novoosnovanoj zemlji, sa ulogom da doprinese povezivanju nauke i prakse, odnosno da omogući razmenu iskustava između istraživača iz Instituta i stručnjaka iz objekata industrije mesa, da informiše o razvoju i mogućnostima industrije mesa u drugim zemljama i da objavljuje rezultate do kojih se došlo, kako u Institutu, tako i drugim naučnim ustanovama. Na stranicama časopisa su objavljivani originalni istraživački radovi i prilozi iz oblasti proizvodnje i prerade svih vrsta mesa od značaja za ishranu ljudi, zatim iz oblasti proizvodnje i prerade jaja, životinjskih masti i ulja, proizvodnje i korišćenja aditiva, ambalažnih materijala i opreme u izradi proizvoda od mesa, kao i iz oblasti mikrobiologije, hemije i kontaminacije mesa i proizvoda od mesa biološkim ostacima. Sadržaj i značaj časopisa i objavljenih radova odražavao je stanje i razvoj Instituta i industrije mesa i drugih pratećih industrija u ovoj oblasti, kao i povezanost sa brojnim naučnim ustanovama u zemlji i inostranstvu. Od 1960. godine u časopisu se objavljuju rezultati osnovnih i primenjenih istraživanja u oblasti biotehničkih nauka, odnosno grana: veterinarstvo, prehrambeno inženjerstvo i biotehnologija. Multidisciplinarnim pristupom svim naučnim i stručnim problemima mobilisani su različiti profili naučnih i stručnih istraživača sa ciljem da se potencijal iz više naučnih oblasti objedinjuje i da se da značajniji doprinos razvoju nauke o mesu.*

*Sa razvojem naučne misli, kako u svetu tako i kod nas, donosi se zakonska regulativa (zakoni, pravilnici i/ili akta), kojom se definišu i regulišu statusi nacionalnih časopisa. Aktom o uređenju nacionalnih naučnih časopisa, svakoj publikaciji na nivou zemlje priznaje se naučni status u postupku kategorizacije predviđene zakonom. Kategorizaciji podležu samo časopisi koji su prethodno položeni u Repozitorijum Narodne biblioteke Srbije u papirnom i elektronskom obliku radi trajnog čuvanja i koji ispunjavaju uslove predviđene Aktom. Centar za evaluaciju u obrazovanju i nauci (CEON) je formiran 2002. godine kao autonomna agencija za istraživanje i razvoj. Osnovna delatnost CEON-a je bila razvoj bibliometrijskih metoda i baza podataka namenjenih evaluaciji i, u isto vreme, promociji istraživačkih rezultata nastalih u Srbiji i u drugim zemljama Jugoistočne Evrope. CEON je bila organizacija koja je obavila sve go-*

*dišnje analize časopisa za potrebe nadležnog ministarstva. Od 2016. godine, Ministarstvo prosvete, nauke i tehnološkog razvoja je angažovalo Matematički institut SANU za izradu, održavanje i publikovanje bibliometrijskog izveštaja o naučnim časopisima za potrebe kategorizacije i rangiranje časopisa u R. Srbiji. U skladu sa tim se i danas obavlja kategorizacija časopisa nacionalnog značaja i vrednovanje naučnog rada. Radovi u časopisima nacionalnog značaja mogu imati sledeće kategorije: M24 — časopis međunarodnog značaja verifikovan posebnom odlukom matičnih naučnih odbora; M51 — vodeći časopis nacionalnog značaja; M52 — časopis nacionalnog značaja i M53 — naučni časopis.*

*Od osnivanja, pa sve do 1988. godine, časopis je izlazio jednom mesečno, zatim dvomesečno, pa tri puta godišnje, a od 2010. godine izlazi dva puta godišnje. Sve je to bila posledica smanjenog broja prispelih radova redakciji časopisa, odnosno rezultat previranja na ovim prostorima. Sve do 2015. godine radovi u časopisu su štampani na srpskom jeziku, sa apstraktima i ključnim rečima, tabelama, grafikonima i slikama na srpskom i engleskom jeziku. Osim toga, pojedini radovi su štampani i na engleskom, nemačkom ili ruskom jeziku. U tom slučaju su njihovi kratki sadržaji, tabele, grafikoni i slike štampani na jeziku na kome je objavljen rad i na srpskom jeziku. Da bi postao dostupan široj naučnoj javnosti, od 2016. godine časopis je počeo da izlazi na engleskom jeziku, sa apstraktima radova, osim na engleskom, i na srpskom jeziku. Časopis je do 2016. godine imao naslov Tehnologija mesa (ISSN 0494-9846), a od 2016. godine Meat Technology (ISSN 2466-4812).*

*Časopis Tehnologija mesa je održavao visok naučni nivo i u vrlo složenim uslovima za razvoj istraživanja i njihov transfer u proizvodnju, preradu i kontrolu mesa i pratio je sve vremenom postavljene zahteve za vrednovanje naučnog rada, u skladu sa razvojem bibliometrijskih metoda neophodnih za uspon na skali za kategorizaciju i rangiranje časopisa na nacionalnom nivou. Časopis je na početku izlaženja, u skladu sa postavljenim ciljevima, ostvarivao kategorije M53 — naučni časopis i/ili M52 — časopis nacionalnog značaja. Od 2009. do 2016. je, uglavnom, ostvarivao kategoriju M51 — vodeći časopis nacionalnog značaja, a od 2017. ima u kontinuitetu kategoriju M24 — časopis međunarodnog značaja verifikovan posebnom odlukom Matičnog naučnog odbora za biotehnologiju i poljoprivredu. Bitno je naznačiti da angažovanje naučnih radnika na pisanju, publikovanju i citiranju radova iz Meat Technology u časopisima sa SCI (Science Citation Index) liste doprineće, ne samo održavanju statusa časopisa u postignutoj kategoriji, već i njegom usponu na viši, međunarodni nivo.*

*Dr Aurelija Spirić, naučni savetnik u penziji*

## *A word from the editor-in-chief*

*Meat Technology is in the group of journals in the field of biotechnological sciences and is ranked 6th of 36 in the Republic of Serbia according to the categorization of the Ministry of Education, Science and Technological Development. The long tradition of publishing of the journal, which turns 60 this year, speaks of the importance of cooperation between the publisher - the Institute of Hygiene and Meat Technology and researchers in the field of meat science, as well as the meat industry. In the hope that we will continue to provide quality scientific papers, both from our country and the region and around the world, we thank all the authors, reviewers and the editorial board of the journal, which for the third year is ranked in the category of National journal of international importance (M24).*

*PhD Vesna Đorđević, spec.,*
*Principal research fellow*

## *Reč glavnog i odgovornog urednika*

*Časopis Meat Technology je u grupi časopisa iz oblasti biotehnoloških nauka i nalazi se na 6. mestu od 36 u Republici Srbiji prema kategorizaciji Ministarstva prosvete, nauke i tehnološkog razvoja. Dugogodišnja tradicija izlaženja časopisa, koji ove godine puni 60 godina, govori o značaju saradnje izdavača, Instituta za higijenu i tehnologiju mesa i istraživača iz oblasti nauke o mesu, kao i proizvođača iz industrije mesa. U nadi da ćemo i dalje obezbeđivati kvalitetne naučne radove, kako iz naše zemlje, tako i regiona i celog sveta, zahvaljujemo se svim autorima, recenzentima, kao i uređivačkom odboru časopisa, koji je već treću godinu zaredom u kategoriji nacionalnog časopisa međunarodnog značaja (M24).*

*Dr Vesna Đorđević, spec.,*
*Naučni savetnik*

ISSN 2466-2852

# meat technology
## scientific journal

Meat Technology Vol. 61 No. 1 P. 1–96 Belgrade 2020

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Based on the opinion issued by Ministry of Education, Science and Technological Development of the Republic of Serbia (No. 413-00-00416/2000-01), this publication is of special interest for the science.

Subscription

Annual subscription rate for aboard is: 100 EUR. Orders should be sent to Institute for Meat Hygiene and Technology, P.O. Box 33-49, Kacanskog 13, 11000 Belgrade, R. Serbia.

Annual subscription rate for Republic of Serbia is 5000.00 RSD. Payments can be made to an account of Institute no 205-7803-56 at Commercial Bank with a note subscription to the journal.

www.journalmeattechnology.com

Computer processing and printing
„Naučna KMD“, Beograd
www.naucnakmd.com
Circulation 150 copies

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PUBLICATION OF THIS JOURNAL IS FINNANCIALLY SUPPORTED BY:
Ministry of Education, Science and Technological Development of the Republic of Serbia

**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 579.67(497.11)*
*ID: 16187145*
*https://doi.org/10.18485/meattech.2020.61.1.1*

*Review paper*

# Current status of mycotoxin contamination of food and feeds and associated public health risk in Serbia

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*A b s t r a c t: Mycotoxins are chemical hazards of microbiological origin, produced mainly by filamentous fungi during their secondary metabolism. The role of mycotoxins has been recognized in the aetiology of a number of diseases, particularly cancers that belong to non-communicable diseases (NCDs). The NCDs have a leading and growing contribution to preventable deaths and disability across the globe. The NCDs are known as chronic diseases, tend to be of long duration and are the result of a combination of genetic, physiological, environmental and behavioural factors. Following the increased interest in health effects caused by synergisms between natural and synthetic contaminants along the food chain, mycotoxin contamination will continue to be an area of concern for producers, manufacturers, regulatory agencies, researchers and consumers in the future. Considering that their presence in food depends strongly on climatic conditions, in Serbia, recent drought and then flooding confirmed that mycotoxins are one of the foodborne hazards most susceptible to climate change. In this article, we review key aspects of mycotoxin contamination of the food supply chain and attempt to highlight the latest trends and projections for mycotoxin reduction from a Serbian perspective.*

***Keywords:*** *mycotoxin, occurrence, public health, SWOT-analysis.*

## Introduction

Food security and safety is one of the major problems currently in protecting human health and the economic development of countries around the world and constitute some of the main challenges of the 2030 Agenda for Sustainable Development. According to data from the World Health Organization (WHO), contaminated food and water cause over 200 human diseases, with up to 30% of the world population suffering from certain types of food- and water-related diseases each year. Among them, 2.2 million people face fatal outcomes, with 1.9 million children dying from these diseases (*WHO,* 2015). Due to continual growth and population migration, an increasing trend in the number of food-borne outbreaks and cases of diseases can be expected. In this context, the responsibility of competent authorities is directed towards the establishment of an effective food safety system, which includes an integrated and well-coordinated longitudinal system from field to table (*Akkerman et al.,* 2010).

Based on data belonging to the Rapid Alert System on Food and Feed (RASFF), in the last ten years, mycotoxins, particularly aflatoxins, were the most commonly reported type of hazard. Data show that 93% of the overall mycotoxin notifications referred to aflatoxins (*RASFF,* 2019). Also, on the basis of data from other agencies, in regard to occurrence, nutritional-health disorders and economic impact, mycotoxins are a very serious problem in the food supply system, especially in developing countries (*WHO,* 2018). Mycotoxins are defined as a structurally diverse group of secondary metabolites, often low molecular-weight compounds, produced by a large number of species of different fungal genera, which can contaminate food commodities along the food chain (*Marin et al.,* 2013). Production of

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secondary metabolites is not directly essential for normal fungal growth, but allows moulds to rapidly colonize the environment and compete with other organisms or inhibit competitor growth and reproduction and, therefore, gives moulds a competitive advantage within complex ecosystems (*Raffa and Keller*, 2019). Although 400 secondary metabolites with toxigenic potential have been identified to date, only about 50 of them have been studied in detail due to their important roles in food safety. The

**Table 1.** Toxicological aspects of the main mycotoxins

| Mycotoxins | Most susceptible crops | Producing fungi | Primary mechanism of action/Health effects | IARC (Group) | Health guidance value | Ref. |
|---|---|---|---|---|---|---|
| AF′s | tree nuts, ground nuts, dried fruits, spices, maize | *Aspergillus flavus, A. parasiticus, A. section Flavi* | Binds to guanine (dnaadduct)/ Carcinogenic, Mutagenic, Teratogenic, Hepatotoxic, Nephrotoxic, Immunosuppressive | 1 | (ALARA principle) $BMDL_{10}$ 170 ng $kg^{-1}$ bw $day^{-1}$ | *EFSA,* 2007, *IARC,* 2012 |
| OTA | cereal grains, coffee, beer, wine, dried fruits, spices, meat products, | *A. ochraceus, P. verrucosum, A. niger A. carbonarius* | Blocks protein synthesis/Mutagenic, teratogenic, neurotoxic, hepatotoxic, Nephrotoxic, immunotoxic | 2B | TWI = 120 ng $kg^{-1}$ bw | *IARC,* 1993, *EFSA*, 2006 |
| FUM′s | maize, maize-based food | *Fusarium proliferatum, F. verticillioides* | Inhibit ceramide synthase/ Esophageal and liver carcinogens, neurotoxic, neural tube defects, genotoxic | | TDI = 1 µg $kg^{-1}$ bw $day^{-1}$ | *IARC,* 1993, 2002, *EF*SA, 2018a |
| STG | coffee beans, spices, nuts and beer | *A. versicolor, A. flavus, A. parasiticus, A. nidulans* | Genotoxicity, carcinogenicity, Liver and kidneys | | PTWI is not established | *IARC*, 2006, *EFSA*, 2013 |
| DON and sum 3-Ac-DON, 15-Ac-DON, DON-3--glucoside | cereal crops, processed grains | *F. graminearum, F. culmorum* | Inhibition of protein synthesis/Gastrointestinal haemorrhagiae, immuno-suppression, dermatosis | 3 | TDI = 1 µg $kg^{-1}$ bw $day^{-1}$ | *IARC,* 1993, *EFSA*, 2014a |
| T-2, HT-2 toxin | | *F. sporotrichioides, F. Poae, F. langsethiae* | DNA damage/Immun depressants mutagenic Gastrointestinal haemorrhaging, neurotoxic | | TDI (T-2+HT-2) = = 0.1 µg $kg^{-1}$ bw $day^{-1}$ | *IARC,* 1993, *EFSA,* 2014b |
| NIV | | *F. graminearum, F. crookwellense, F. nivale* | Inhibit protein and DNA synthesis, immunosuppresive | | TDI = 1.2 µg $kg^{-1}$ bw $day^{-1}$ | *IARC,* 1993, *EFSA*, 2014b |
| ZEA | maize, cereal crops | *F. graminearum, F. culmorum, F. equiseti, F. cerealis, F. verticillioides,* | Binds to mammalian estrogen receptor/ Hyperestrogenism, Reproductive disorders, infertility, early pubertal changes | | TDI = 0.25 µg $kg^{-1}$ bw $day^{-1}$ | *IARC,* 1993, *EFSA*, 2014a |
| PAT | apples and apple-derived foods | *Penicillim expansum, Bysochlamis nívea, A. clavatus* | DNA and RNA synthesis inhibition/ gastrointestinal symptoms, neurotoxic, immunosuppressive, mutagenic | | TDI = 0.4 µg $kg^{-1}$ bw $day^{-1}$ | *IA*RC, 1993, *EFSA*, 2006 |

**Legend:** AF′s-Aflatoxin (B1,B2,G1,G2,M1); OTA-Ochratoxin A; FUM′s-Fumonisin (B1, B2); STG-Sterigmatocystin; DON-deoxynivalenol; NIV-nivalenol; ZEA- Zearalenone; PAT-patulin; Group 1, carcinogenic to humans; Group 2A, probably carcinogenic to humans; Group 2B, possibly carcinogenic to humans; Group 3, not classifiable as to its carcinogenicity to humans. ALARA principle- As low as reasonably achievable, TDI: tolerable daily intake, PTWI, provisional tolerable weekly intake, Reference Point

most important agro-economic and public health classes of mycotoxins are aflatoxins, ochratoxin A (OTA), zearalenone (ZEA), trichothecenes (TCTs), fumonisins (FUMs), patulin (PT), *Alternaria* toxins and ergot toxins. They are produced by species of *Fusarium, Penicillium, Aspergillus, Claviceps* and *Alternaria* (*Liew and Mohd-Redzwan,* 2018; *Rai et al.,* 2019). Some of them can produce more than one mycotoxin, and some mycotoxins are produced by more than one fungal species (*Marin et al.,* 2013) (Table 1). Their common co-occurrence at low levels in food and feed presents a threat to human and animal health, inducing major economic losses for farmers, industry, international trade and society.

Besides these, some other mycotoxins such as "modified mycotoxins", "masked mycotoxins", and new, emerging mycotoxins (moniliformin, enniatins, beauvericin and fusaproliferin) are also the subjects of notable attention in on-going investigations (*Jajic et al.,* 2019). The term *modified mycotoxins* refers to any mycotoxin with a structure that has been changed in the course of some chemical/biochemical reaction by plants, animals, fungi or by processing (*Rychlik et al.,* 2014). *Masked mycotoxins* are a group of mycotoxins produced during some detoxication reactions implemented by plants in an attempt to neutralize native mycotoxins (*Khaneghah et al.,* 2018). Considering that modified mycotoxins are usually not detected by commonly used analytical methods, only limited data are available on their occurrence in crops. Thus, their impact on food safety may be even more relevant than it currently seems.

Most mycotoxins are relatively heat-stable in the conventional food processing temperature range (cooking, baking, frying, roasting), and thus, they remain in the final product (*Udovicki et al*., 2018; *Carballo et al.,* 2019). Therefore, human contamination with mycotoxins can occur directly through the consumption of foods containing mycotoxins or indirectly (carry-over) through consumption of mycotoxins and/or their metabolites from animal tissues, milk and eggs (*Zadravec et al.,* 2020: *Milicevic et al.,* 2014) Ingestion of mycotoxin-contaminated food/feed results in a disease (mainly subclinical) known as mycotoxicosis. Depending on the mycotoxin's toxicity, its concentrations in food, the duration of exposure, and the age and nutritional status of the at-risk individual, mycotoxin health-related risks range from acute to chronic (mutagenic, teratogenic, carcinogenic) manifestations in both animals and humans (*Datsugwai et al.,* 2013; *Richard,* 2007). In 1993, the International Agency for Research on Cancer (*IARC*, 1993) evaluated the carcinogenic potential of aflatoxins OTA, TCT, ZEA and FUMs and in 2012 re-evaluated aflatoxin M1 (AFM1) and carcinogenicity (*IARC,* 2012; *Ostry et al.,* 2017). Naturally-occurring aflatoxins (AFB1, AFB2, AFG1, AFG2 and AFM1) were classified as carcinogenic to humans (Group 1), while OTA and FUMs were classified as possible carcinogens (Group 2B). TCT, ZEA and PT, however, were not classified as human carcinogens (Group 3) (Table 1).

The economic losses and health hazards posed by mycotoxin contamination of food and feed are a huge challenge, and this is especially severe in developing countries (*Milicevic et al.,* 2019a). The economic impact of mycotoxin contamination includes loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce the severity of the mycotoxin problem (*Zaki et al.,* 2012). However, these losses represent only part of the economic losses. The most information on economic impact is available for the United States, where the cost of mycotoxin contamination to the U.S. economy was estimated to be between $2 billion and $3 billion per year, depending on the year (*Sassi et al*., 2018). Accordingly, in order to protect consumer health and to reduce economic losses, surveillance and monitoring of mycotoxins in food and feed, and implemented in public health programs, have become major objectives for producers, regulatory authorities and researchers in Serbia (*Milicevic et al.,* 2016).

## Mycotoxin Studies in Serbia

Since the discovery of aflatoxins in 1960, a number of studies on the occurrence of mycotoxins in Serbia have been conducted (*Ozegivic and Aganovic*, 1963; *Popovic et al.,* 1968; *Kordic,* 1986). These older surveys mainly followed diseases in domestic animals, leading to further research to answer questions related to the human health relevance. In general, from 2006 to date, more sensitive analytical techniques and research concepts were employed, leading to great progress in mycotoxin research. This resulted in large data pools and new insights into mycotoxin prevalences, concentrations, mitigation measures and risk assessments. Recently, several studies have assessed the effects of climate change on food safety, including the occurrence of mycotoxin-producing fungi and mycotoxins in foods/feeds. These data clearly show that mycotoxin

contamination is becoming a serious problem in Serbia because of the negative public health effects, but particularly because of the negative effects on the economy and trade.

Therefore, the aim of this review is to overview studies published between 2011 and 2019 about the incidence of mycotoxins in Serbia, and we attempt to highlight the latest trends and projections for mycotoxin reduction from the Serbian perspective.

## Impact of climate factors in Serbia on mycotoxin production

Fungal colonization and/or mycotoxin production is influenced by various ecological and environmental factors (*Tola and Kebede,* 2016). *D'Mello and MacDonald* (1997) categorized these factors as physical (moisture, RH, temperature and mechanical damage), chemical (carbon dioxide, oxygen, composition of substrate, pesticide and fungicides), and biological (plant variety, stress, insects and spore load). The biological factors have been further subcategorized to include intrinsic factors (including fungal species, strain specificity, strain variation and stability of toxigenic properties). Moreover, temperature (t), relative humidity (RH), rainfall (R) and grain water activity ($a_w$) are the most important ecological factors that modulate fungal growth and mycotoxin production pre-harvest or during storage (*Palumbo et al.,* 2020). Therefore, drought (and the RH related to it) is a modulator of mycotoxin contamination that is expected to be more frequent in the future, depending on geography. Climate change seems to be another important factor affecting mycotoxin contamination of foods and feedstuffs (*Milicevic et al.,* 2019b).

Traditionally, the Serbian climate is considered as a warm-humid continental or humid subtropical climate, with more or less pronounced local characteristics. Recent decades have seen an increasing occurrence of extreme weather events. In the 2012 production year in the major part of the country, the hottest and driest period coincided with the most important generation phases of spring crops. This climatic event had not occurred previously in Serbia, and it caused substantial damage and losses in agricultural crop production. In addition, a high average frequency of *Aspergillus spp*., particularly *Aspergillus flavus* (which is xerophilic) and *Aspergillus niger*, on analyzed grain (95.3%) (*Levic et al.,* 2013), followed by the high incidence of aflatoxins in maize and consequently in feed and milk, were also attributed to the hottest and driest period (*Milicevic et al.,* 2017, 2019b). Therefore, several RASFF notifications related to aflatoxin levels above the MPLs in maize from countries from South-East Europe were issued at the end of 2012 and continued on in the first months of 2013 (*RASFF,* 2013).

Before 2008, *Aspergillus spp*. in Serbian grain occurred mostly at low frequency with an incidence of 3% to 16% (*Levic et al.,* 2013), but the very high temperatures and extreme drought in 2012 caused an outbreak of aflatoxins in epidemic proportions. These findings indicate that changes in environmental temperature influence the expression levels of regulatory genes (*aflR* and *aflS*) and aflatoxin production in *A. flavus* and *Aspergillus parasiticus* (*Schmidt-Heydt et al.,* 2010). *Gallo et al*., (2016) reported that regulatory genes *aflR* and *aflS* were highly expressed at 28°C, while the lowest expression was observed at 20 and 37°C. Generally, the optimum conditions for AFB1 production were 30–35°C at 0.95 $a_w$, and 25–30°C at 0.99 $a_w$, while no fungal growth or AFB1 production was reported when the temperature fell below 20°C ($a_w$ 0.90 and 0.93) or when the temperature was higher than 40°C (*Liu et al.,* 2017). However, the study revealed that low levels of OTA-producing *Aspergillus* were present on Serbian feed/food compared to levels in the tropical countries.

In contrast, 2014 weather conditions were significantly different to those in 2013 and 2015. Furthermore, 2014 was characterized by a significantly higher number of rainy days than normal, and thus, in most of Serbia, precipitation was at a historical maximum during the 2014/2015 production year. During the vegetation period (April-September) in 2014, an average of 700 mm (400–1200 mm) of rain was recorded in Serbia, which was the worst season in the last 45 years. This consequently induced a high average moisture content in harvested maize and wheat kernels (>12% w/w), followed by co-occurrence of multiple mycotoxin-producing moulds in cereals, mainly *Fusarium* and *Penicillium* (*Jajic et al.,* 2017; *Kos et al.,* 2020). Recently, a report on the occurrence of mycotoxins in maize harvested in Northern Serbia in the period that included seasons with extreme drought (2012), hot and dry conditions (2013 and 2015) and extreme precipitation (2014) revealed significant differences in the incidences of AF, OTA, ZEA and FUM in the different investigation years (*Kos et al.,* 2020). Results showed that FUMs were detected annually with very high prevalences (found in from 76% to 100% of maize). AFB1 was detected in 94% and 90% of maize from 2012 and 2015, respectively, while during the 2014

**Table 2.** Cereal production (yields) in Serbia during 2014–2019 (SYRS, 2019)

| Year | Maize | | Wheat | | Barley | | Oats | |
|---|---|---|---|---|---|---|---|---|
| | Total tons* | t/ha | Total tons* | t/ha | Total tons* | t/ha | Total tons* | t/ha |
| 2014 | 7.95 | 7.5 | 2.38 | 3.9 | 3.23 | 3.6 | 7.49 | 2.4 |
| 2015 | 5.45 | 5.4 | 2.43 | 4.1 | 3.62 | 3.8 | 8.82 | 2.7 |
| 2016 | 7.37 | 7.3 | 2.88 | 4.8 | 3.95 | 4.3 | 8.13 | 3.0 |
| 2017 | 4.02 | 4.0 | 2.27 | 4.1 | 3.05 | 3.3 | 6.95 | 2.4 |
| 2018 | 6.96 | 7.7 | 2.94 | 4.6 | 4.10 | 3.9 | 7.47 | 2.9 |
| 2019 | 7.34 | 7.6 | 2.53 | 4.4 | 3.73 | 3.7 | 5.62 | 2.5 |

**Legend:** *–$10^6$

production year, DON, ZEA and their derivatives were detected in 100% of the maize studied. OTA was the most predominant in maize (25% of maize contained this mycotoxin) from 2012. In this context, weather conditions that occurred in the four-year period had a significant influence on the occurrence of the examined mycotoxins in maize (*Kos et al.,* 2020). Also, a similar feed survey performed during the same four-year period (2012–2015) highlighted the problem of high levels of co-contamination with a number of different mycotoxin-producing species (*Krnjaja et al.,* 2017). Most of the maize sampled (and based on average values) contained *Fusarium* (92.2%), followed by species of the genera *Aspergillus* (80.8%) and *Penicillium* (48.7%) (*Krnjaja et al.,* 2017). Analysis of publications in recent years indicated that weather conditions in 2014 and 2016 were much more favourable for some *Fusarium* species than for *Aspergillus* species and aflatoxin synthesis (*Kos et al.,* 2017). In 2017, agro-meteorological conditions were unfavourable for many agricultural crops. During the production part of the year, the maximum daily temperatures (>35°C) were above the annual average. Furthermore, drought was the predominant factor that caused the greatest damage, especially to maize, and when compared with 2016, the total 2017 maize production was decreased by 45.5% (Table 2). The next two years (2018 and 2019), particularly 2019, was the hottest year in the history of meteorological measurements in Serbia. According to a report by the Republic Hydrometeorological Service of Serbia, 13 of the 15 hottest years in Serbia were registered after 2000 (the period considered was 1951 to 2019) (*RHSS*, 2020).

The co-occurrence of high air temperatures (up to 40°C) and heavy rainfall with high RH (up to 80%) within the same year is an emerging weather pattern in Serbia. These conditions before cereal harvest and during storage play an important role in mycotoxin occurrence. Under such changed climate conditions, the occurrence of toxigenic fungi and consequent co-contamination of cereals with mycotoxins is a considerable hazard we should bear in mind.

## Incidence of mycotoxins in Serbia

Mycotoxicoses in humans or animals are characterized as food or feed related, noncontagious, nontransferable, noninfectious, nontraceable to microorganisms other than fungi, and do not show immunogenicity (*Zain*, 2011). The symptoms of mycotoxicosis depend on the type of mycotoxin, the chemical properties of the agents such as the ability to penetrate cell membranes, the intake route, the duration of exposure, the concentration, and the presence of other mycotoxins and pharmacologically active substances, as well as the health, exposure to infectious agents, age and sex of the exposed individual (*Williams et al.,* 2011). Clinical symptoms usually subside on removal of contaminated food or feed. Despite efforts to control fungal contamination, multi-mycotoxin contamination is of great concern and seems to be "the most important chronic dietary risk factor, higher than synthetic contaminants, plant toxins, food additives, or pesticide residues" (*Lee et al.,* 2017; *Williams et al.,* 2011). On a global level, 30% to 100% of food and feed samples are

contaminated by mycotoxins. According to results from the World Mycotoxin Report, FUMs are still abundant at high concentrations in raw commodities. Regional examples of mycotoxin incidence indicated that in Europe, the most prevalent mycotoxin is DON (65%), followed by FUMs (56%), ZEN (44%), aflatoxins (31%) and OTA (27%) (*Pinotti et al.,* 2016; *Vila-Donat et al.,* 2018). *Fusarium* mycotoxins (FB1, FB2, DON, ZEN) and OTA were mostly detected in the maize harvested in 2018 from European countries.

Extreme weather events in Serbia pose one of the greatest risks for contamination of cereals such as wheat, maize, barley and oats by various species of toxigenic fungi and their related mycotoxins (*Udovicki et al.,* 2018; *Udovicki et al.,* 2019a). Serbia has a largely agrarian economy, and thus, mycotoxin contamination of agricultural products has had a strong negative impact on Serbian trade, especially with the European Economic Community markets. Cereals, particularly maize, are one of the major feedingstuffs in the world because of their importance as a main source of energy and protein in animal feeding (*Jovanovic et al.,* 2018). In the last few years in Serbia, cereal production showed year-to-year variations depending on the climate conditions (Table 2). However, maize is one of the most important agricultural products in the country, both by its production and by the profit it generates in foreign trade (*SYRS*, 2019; *Index Mundi*, 2018). A high portion of this production is consumed locally (4.2 and 0.2 to 0.3 million tons for animal feed and for human consumption, respectively) and the remainder is exported to foreign countries (*Maslac,* 2017).

Data on the occurrence of mycotoxins are extremely important to determine the risk posed by mycotoxins both to humans and animals, and moreover, this is one part of risk assessment that contributes to new and effective regulations, improvement of laboratory facilities etc. This is particularly the case in vulnerable countries that are prone to mycotoxin contamination, such as Serbia. In Tables 3–5, an overview of updated information on mycotoxin occurrence in different commodities since the start of the aflatoxin crisis in Serbia is shown. Most of the recently published papers deal with aflatoxins, DON, FUMs, ZEA, TCT and OTA mycotoxins, on which there are more data. Also of great concern for risk assessments and as possible hazards for human health are the emerging mycotoxins such as moniliformin (MON), enniatins (ENs), beauvericin (BEA) and fusaproliferin (FUS), which are under consideration. We emphasize that the data presented in the tables were obtained using different methodologies, with distinct sensitivities and accuracies, as well as sampling methods and number of analyzed samples.

*Aflatoxins*

Aflatoxins are difuranocoumarin derivatives produced by a polyketide pathway by many strains of *A. flavus*, *A. parasiticus*, and the rare *Aspergillus nomius*, which contaminate agricultural commodities (*De Ruyck et al.,* 2015). To date, nearly 20 different types of aflatoxins have been identified, the six predominant ones being aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), AFM1 and aflatoxin M2 (AFM2) (based on their blue or green fluorescence under UV light). AFB1 is identified as the most potent naturally occurring carcinogen in this group, and can cause serious health issues such as growth retardation, genotoxic, carcinogenic, and teratogenic effects for both humans and animals (*Zhou et al.,* 2019), while the other compounds have lower toxicity. *IARC* (2012) classify AFB1, AFB2, AFG1, AFG2 and AFM1 as Group 1 carcinogens, emphasizing their explicit carcinogenicity to humans. The no observed adverse effect level is not applied to genotoxic carcinogens, and therefore, no threshold is assigned to AFB1 (*IARC,* 1993). Aflatoxins are rapidly absorbed and metabolized, primarily in the liver by cytochrome P450 enzymes to reactive epoxides, which can react with cellular targets (e.g., DNA, RNA and proteins), forming covalent bonds (*Carter et al.,* 2019). The rate of metabolism and the type of metabolic products determine differences in species susceptibility to aflatoxins. Most of the metabolic products, such as AFM1 and aflatoxin Q1 (AFQ1), are less toxic than the parent AFB1, but aflatoxin B1–8,9-expoxide (AFBO) is the most toxic metabolite form and is responsible for carcinogenic effects, especially in the liver (*WHO*, 2018). The hepatocarcinogenicity of aflatoxins is mainly due to lipid peroxidation that disrupts transcription and translation to DNA (*Zhang*, 2015). Thus, epoxidation is generally considered as metabolite activation, while hydroxylation, hydration, and demethylation are considered metabolic detoxications. Aflatoxin adducts in urine and blood are reliable biomarkers of aflatoxin exposure (*Al-Jaal et al.,* 2019).

AFM1 is a less mutagenic and carcinogenic (2 to 10%) hydroxylated metabolite of AFB1, and is excreted into the urine and milk of mammals after they ingest foods contaminated with AFB1 (*Kumar et al.,* 2017). The average conversion value was

2.5%, although a direct relationship between the carry-over rate and the milk yield, with a maximal 6.2% carry-over rate, was found (*Walte et al.,* 2016). In dairy cows, excretion of AFM1 occurs in as little as 12 to 24 h and up to 2 to 3 days in milk (*Peles et al.,* 2019). AFM1 clearance from cow milk depends on several factors, but mainly on the amount of AFB1 ingested and the duration of mycotoxin consumption, with excretion by cows occurring for a variable period of about 5 to 7 days from the cessation of AFB1 consumption (*Peles et al.,* 2019). Approximately 95% of AFB1 metabolites excreted in milk are in the form of AFM1, although AFM2, AFG1, and AFB2 are also reported (*Ostry et al.,* 2017). A recent review (*Sengling Cebin Coppa et al.,* 2019) of the occurrence of mycotoxins in breast milk, fruit products and cereal-based infant formula found that mycotoxins such as AFM1 and OTA have been reported in human breast milk and in infant formulae in different concentrations globally, while the reported levels in some European, African and Asian countries indicates high exposure levels, which can potentially result in adverse health effects in infants and present a serious public health problem.

Various investigations conducted in Serbia in the last decade have revealed a significant presence of aflatoxins in maize (*Kos et al.,* 2017; *Udovicki et al.,* 2018). The most recent research from the same authors (*Kos et al.,* 2020) was conducted to evaluate the incidence of the mycotoxins aflatoxin, ZEN, OTA, DON and their metabolites in maize between 2012 and 2015. This study confirmed that conditions of extreme drought in 2012 had a great influence on the presence of AFB1 (94% of maize was contaminated). In comparison to maize from 2012, the percentages of contaminated maize in 2013 (33%) and 2015 (90%) were lower, as were the mean aflatoxin levels detected. In 2014, extremely rainy conditions recorded during the maize growing season were unfavourable for the growth of some *Aspergillus* species and for aflatoxin synthesis. Although, AFB1 was detected in maize in 2015 with a high prevalence (90%), the mean concentration of AFB1 (8 μg $kg^{-1}$) was significantly lower than the mean concentration (44 μg $kg^{-1}$) detected in maize from 2012 (*Kos et al.,* 2020).

Due to the severity of the maize contamination, elevated concentrations of AFM1 were found in milk countrywide, with a large percentage of milk samples (68%) being non-compliant according to the European Union maximum residue limit (EU-MRL 0.05 μg $kg^{-1}$) (*Stefanovic*, 2014). As a relatively stable compound during pasteurization and sterilization (*Peng et al.,* 2018), AFM1 is more concentrated in curd and cheese than in the milk itself that was used for cheese-making. Furthermore, 2.5–3.3-fold and 3.9–5.8-fold higher concentrations of AFM1 were recorded in soft and hard cheeses, respectively, than the AFM1 concentrations found in milk from which cheeses were made (*Filazi and Sireli*, 2013). As infants depend on milk as a basic food, it is extremely important to control the level of aflatoxins in milk. The results obtained from a systematic review (2015–2018) showed year-to-year variations of AFM1 prevalence, and average contamination levels in the analyzed milks were significantly different ($P < 0.001$) (*Milicevic et al.,* 2017; 2019b). Likewise, AFM1 incidence has shown an interesting periodic fluctuation over the survey period. Results of this study are consistent with reports from other countries stating that the prevalence of AFM1 in raw milk was significantly higher ($P < 0.05$) during spring than in summer and autumn. According to the report (*Milicevic et al.,* 2017; 2019b), improper storage conditions, particularly during winter and spring, combined with the initial fungal contamination in the fields had a great impact on AFB1 levels in cattle feed, and consequently on AFM1 occurrence in milk. Moreover, the findings of this study showed the increase in AFM1 occurrence was significantly positively correlated with air temperature and annual RH (*Milicevic et al.,* 2017; 2019b). Although the incidence of AFM1 is currently tending to decrease year by year in Serbia, it is evident that the incidence could increase with a rise in global temperatures. Overall, AFM1 has proved to be a great public health concern in Serbia and should be considered as a high priority for risk management actions.

### *Sterigmatocystin*

Sterigmatocystin (STC) is a polyketide mycotoxin structurally related to AFB1, and is produced by several fungal species, including *A. flavus, A. parasiticus, Aspergillus versicolor* and *Aspergillus nidulans*, of which *A. versicolor* is the most common source. During the last 30 to 40 years, only a limited number of surveys on the occurrence of STC in different foods and feed were carried out. Therefore, data are too limited to conduct a reliable human dietary exposure assessment. The toxin has been reported in grains, nuts, green coffee beans, spices, beer and cheese. The carry-over of STC and/or its metabolites from feed to animal products such as meat and eggs, leads to an exposure of low health

concern (*EFSA,* 2013). Liver and kidneys are the target organs of acute toxicity. STC is hepatotoxic in poultry and pigs, and nephrotoxic in poultry. The carcinogenic potency of STC is approximately three orders of magnitude lower than that of AFB1. Due to the absence of exposure data for the European population, the margin of exposure (MOE) approach for substances that are genotoxic and carcinogenic cannot be applied for STC, and, therefore, the European Food Safety Authority's CONTAM Panel could not characterize the risk STC has for human health (*EFSA,* 2013). Furthermore, *IARC* (1987) concluded that STC is possibly carcinogenic to humans (in the group 2B carcinogens).

*Ochratoxin A*

Ochratoxin (OTA) is produced by fungi of the genera *Aspergillus* and *Penicillium* contaminating a wide range of commodities, including staple food crops and beverages such as beer and wine. OTA comprises a dihydrocoumarin moiety linked to a molecule of L-β-phenylalanine via an amide bond. OTA is one of the most relevant mycotoxins, with great public health and agro-economic significance due to the toxin's confirmed nephrotoxic, genotoxic, neurotoxic, immunotoxic, embryotoxic and teratogenic effects, and its suspected carcinogenicity (*Pfohl-Leszkowicz*, 2012; *IARC*, 2014; *Malir et al.,* 2016; *Koszegi and Poor,* 2016).

Hence, OTA has been studied more often than other mycotoxins in our region. In temperate countries like Serbia, OTA is produced by *Penicillium verrucosum* and associated with contamination of several foodstuffs, such as cereals, wine, eggs, pork meat and some herbs. A recent study detected OTA in 13 (25%), 1 (2%) and 9 (18%) maize samples from 2012, 2013 and 2015, respectively (*Kos et al.,* 2020). The highest OTA prevalence detected (25% of maize examined contained OTA) was from 2012, which means the prolonged drought provided the most favourable conditions for the growth of some *Aspergillus* species and synthesis of OTA (*Kos et al.,* 2020). These findings are in line with trends of OTA occurrence in Serbian cereal grains or different types of flour as observed in similar survey (*Torovic et al.,* 2018a). Currently in Serbia, research is not focused on OTA, since research attention is oriented toward aflatoxins and fusariotoxins. However, under climate conditions with elevated temperatures, there is an important question regarding which fungal species is responsible for OTA contamination of cereals. Not surprisingly, low levels of OTA-producing *Aspergillus* are present in agricultural products. OTA is a heat-stable compound (stable at temperatures >250°C) that is not destroyed by common processing treatments (*Marin et al.,* 2013). Animal feeds are usually contaminated by OTA due to improper storage conditions during production and transportation (*Krnjaja et al.,* 2014; *Radulovic et al.,* 2013).

Unlike the other monitored mycotoxins, OTA has the potential to bioaccumulate in animals' bodies, and thus, contamination of animal feeds by OTA results in the presence of residues in edible tissues (kidneys and liver, in particular), which are often used in the meat industry (*Milicevic et al.,* 2014). OTA levels in cow milk are low due to efficient degradation of OTA in the rumen. The most important contributors to chronic dietary exposure to OTA were processed meat, cheese and grains and grain-based products. Historically, consumption of pork has been a significant source of human exposure to OTA in Serbia. A recent OTA survey of pig kidneys originating from different regions of Vojvodina (Serbia's northern province) revealed that 14 of 95 (14.7%) kidneys were contaminated with OTA at levels between 0.10–3.97 μg $kg^{-1}$ (average 1.36 μg $kg^{-1}$) (*Polovinski et al.,* 2019). Considering the differences in the occurrence of OTA in edible tissues reported in previous research (*Milicevic et al.,* 2011), and in spite of the fact that levels of OTA in edible tissues did not pose immediate hazards for human health, it seems that stored feed should be regularly monitored to detect unexpected OTA residues. This is because of the bioaccumulation of OTA in humans.

The target organ for OTA toxicity is the kidneys, and initial interest in this group of toxins was as a causative agent of porcine nephropathy (*Milicevic et al.,* 2009a). OTA has been hypothesized to cause oxidative damage to DNA, leading to mutagenesis and potential carcinogenesis. Subsequently, OTA has been associated with human disorders, chronic interstitial nephropathy and Balkan endemic nephropathy in the former Yugoslavia, associated with urothelial cancer *(Pavlovic*, 2013). OTA is classified as a possible human carcinogen (group 2B) by IARC (1993) on the basis of sufficient evidence of carcinogenicity in animal models, but insufficient evidence from human studies. Based on the last assessment by the Scientific Committee on Food (SCF), a tolerable weekly intake (TWI) of 120 ng $kg^{-1}$ body weight (bw) was derived for OTA (*EFSA,* 2010).

*Fumonisins*

These compounds (FUMs) are predominantly produced in maize and maize products by *Fusarium proliferatum* and *Fusarium verticillioides* (formerly *Fusarium moniliforme*) (*Uegaki et al.,* 2012). Although 28 FUM analogues have been identified, fumonisin B1 (FB1) is the most studied and most toxic of the metabolites, which have a long chain hydrocarbon unit (similar to that of sphingosine and sphinganine) playing a role in their toxicity. The toxicity of FUMs largely reflects their ability to disrupt sphingolipid metabolism by inhibiting the enzyme ceramide synthase, an enzyme responsible for the acylation of sphinganine and sphingosine. These lipids play an important role at the cellular level, maintaining cell membrane structure, and enhancing cell interaction and extracellular interaction (*Wan et al.,* 2013) According to results from the World Mycotoxin Survey, FUMs are still abundant at high concentrations in raw commodities. Results from a global survey indicated that FUMs are the most common mycotoxins, found in 64% of all analyzed maize samples (*Biomin*, 2019).

As demonstrated in two studies on FUM contamination of maize in Serbia, the percentage of contamination varied from 51% to 100%, depending on the harvesting season (2005 to 2014), as did the mean FUM concentration in positive maize (from 0.227 to 35.760 mg $kg^{-1}$) (*Krnjaja et al.,* 2015; *Jaksic et al.,* 2019). A similar regional pattern of contamination was confirmed by a recent report, which concluded that FUMs were the most prevalent mycotoxins found in contaminated maize during 2012 to 2015 (*Kos et al.,* 2020). These results can be explained by the heavy total rainfall during the maize harvest in 2014 and mild winter during 2015, as well as uncontrolled temperature and RH in the warehouses, which caused the intensive development of toxigenic mould and particularly increased the content of TCT mycotoxins in stored kernels.

Consumption of FUMs has been associated with elevated human oesophageal cancer incidence in high-risk populations (*Waskiewicz et al.,* 2012; *Chen et al.,* 2018). Because FB1 reduces uptake of folate in different cell lines, FUM consumption has been implicated in neural tube defects in human babies. FUMs can also induce hepatotoxicity, nephrotoxicity and renal carcinogenesis (*Kamle et al.,* 2019). In animals, FUMs cause equine leukoencephalomalacia (ELEM) a brain disease in horses, porcine pulmonary oedema in swine and liver and kidney cancer in multiple rodent species and strains. Hence, FB1 is listed as a Group 2B carcinogen (*IARC*, 1993), and a recent evaluation by *EFSA* (2018) established a group tolerable daily intake (TDI) of 1μg $kg^{-1}$ bw $day^{-1}$ based on increased incidence of megalocytic hepatocytes found in a chronic study with mice.

*Zearalenone*

Zearalenone (ZEA) is one of the most prevalent nonsteroidal oestrogenic mycotoxins produced by *Fusarium* genera such as *Fusarium graminearum, Fusarium culmorum, Fusarium crookwellense, Fusarium semitectum* and *Fusarium equiseti*, *w*hich are distributed worldwide (*Rai et al.,* 2019). ZEA and its major alcohol metabolites, α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), α-zearalanol (α-ZAL, zeranol) and β-zearalanol (β-ZAL, tarelanol) share structural similarity with the sex hormone, 17β-estradiol, and can be found naturally or as result of metabolism of humans and animals (*Danicke,* 2015). Therefore, ZEA and its metabolites with their binding affinities for hepatic, uterine, mammary, and hypothalamic oestrogen receptors play an important role in reproductive disorders in human and animals (*Poor et al.,* 2015). Swine are the most sensitive domestic animal species, followed by ruminants, while birds are the most resistant species. In the agroecological conditions in Serbia, *Fusarium*, particularly *F. graminearum*, has relatively high potential for the synthesis of ZEA (*Stepanic et al.,* 2011), and hence, ZEA is one of the most common contaminants of cereals and their products (*Milicevic et al.,* 2009b; *Torovic,* 2018b). Moreover, rainy conditions during the maize growing season, besides being favourable for production of DON and its derivatives, were also favourable for synthesis of other fusariotoxins, ZEA, α-ZOL, β-ZOL and ZEA-S (*Kos et al.,* 2020). ZEA and ZEA-S were detected in 100% of examined maize samples in 2014, while β-ZOL was detected in 96% and α-ZOL in 61% of maize. In 2012, 2013 and 2015, ZEA was detected in 12%, 37% and 53%, respectively, of the maize examined, with significantly lower mean concentrations than occurred in 2014 (*Kos et al.,* 2020). A number of studies (described in *Nesic,* 2015) suggest that *Fusarium* toxicoses are one of the major threats to farmers in Serbia. Consumption of contaminated feed by dairy cows did not result in carry over of ZEA or its metabolites in milk at levels hazardous to human health (*Flores-Flores et al.,* 2015).

Taking into account its prevalence and heat stability (up to 160°C), ZEA cannot be completely removed from the food chain. IARC found limited evidence of ZEA carcinogenicity in animal models,

classifying it together with DON in Group 3 carcinogens. In 2000, the SCF established a TDI of 0.2 μg $kg^{-1}$ bw $day^{-1}$ for ZEA. However, in 2011 the SFC concluded that a TDI of 0.25 μg $kg^{-1}$ bw $day^{-1}$ should be established based on recent data in the most sensitive animal species (*EFSA,* 2014a).

*Trichothecenes*

Several fungal genera are capable of producing trichothecenes (TCT), but most of them are produced by *Fusarium spp*. The TCT mycotoxins comprise a vast group of more than 100 fungal metabolites with the same basic structure, affecting several major cereal crops including oats, barley, maize and wheat. Examples of type A TCT include T-2 toxin (T-2), HT-2 toxin (HT-2) and diacetoxyscirpenol (DAS) (*Escriva et al.,* 2015). Fusarenone-X (FUX), DON, and nivalenol (NIV) are some of the common naturally occurring type B TCTs.

T-2 toxin has received particular attention due to its specific effects on humans and animals, including cardiotoxicity, hepatotoxicity, digestive toxicity, neurotoxicity, and other multisystemic toxicities (*Escriva et al.,* 2015). However, reproductive disorders are the principal deleterious effects of T-2 toxin (*Schuhmacher-Wolz et al.,* 2010). At the cellular level, the main toxic effect of TCT mycotoxins appears to be a primary inhibition of protein synthesis, interfering in the initiation, elongation and termination steps, and secondary destruction of DNA and RNA synthesis. The toxins bind to peptidyl transferase, which is an integral part of the 60S ribosomal subunit. However, studies on cellular dysfunctions and mitochondrial fusion/fission suggest T-2 toxin could inhibit mitochondrial dysfunction and promote mitochondria fragmentation accompanied by deficiency of ATP supply and oxidative stress, which could greatly contribute to the disorder of mitochondrial dynamic balance (*Yang et al.,* 2020). Furthermore, exposure to TCNs can lead to multiple adverse health effects such as vomiting, anorexia, headache, intestinal haemorrhage and oxidative stress (*Alshannaq and Yu,* 2017). Pigs and horses are among the animals that are most sensitive to T-2, the major effects of which are immunological and haematological in nature (*Fang et al.,* 2019).

Although the group of TCTs has been thoroughly studied worldwide, in Serbia, more intensive studies on DON were initiated after 2005. Recently, a very comprehensive study was conducted in a four year period (2012–2015) in order to follow annual climate conditions and impact on mycotoxin incidences (*Kos et al.,* 2020). The prevalence of DON and its derivatives in maize from the 2014 growing season, which was described as extremely rainy and wet, was very high, since DON, DON-3G and 15-ADON were detected in 100%, 100% and 98% of analyzed maize, respectively. Contrary to this, in the hot and dry conditions recorded in 2012, 2013 and 2015, DON was detected in 63%, 35% and 63% of examined maize, respectively. Moreover, mean concentrations in examined maize from 2012, 2013 and 2015 were significantly lower than the mean detected concentration in 2014 (*Kos et al.,* 2020). Similarly, *Jajic et al.* (2017) investigated DON in maize from two harvest seasons in Serbia (2014 and 2015), and found the presence of DON in maize was related to the different weather conditions that prevailed during the two harvest seasons (*Jajic et al.,* 2017). It is evident high temperatures and high air humidity during the vegetation period of cereals promote *Fusarium* producers of DON and the consequent high contamination levels of cereals in Serbia. Although the maximum permitted level of DON in food is specified by regulation in Serbia, and consumption of maize and wheat has increased, there are still insufficient data about the daily intake of *Fusarium* toxins by consumers in Serbia.

In 2003, IARC designated DON, NIV, T-2 and HT-2 toxins as Group 3 (not classifiable) human carcinogens due to inadequate evidence of animal carcinogenicity, and lack of investigation in humans. TDIs of 1 μg $kg^{-1}$ bw $day^{-1}$ and 1.2 μg $kg^{-1}$ bw $day^{-1}$ were established for DON and NIV, respectively (*EFSA,* 2014a; 2014b). Recently, the SCF concluded that a full TDI of 0.1 μg $kg^{-1}$ bw $day^{-1}$ for the sum of T-2 and HT-2 toxins be established (*EFSA*, 2014b).

*Patulin*

Patulin (PAT) is a mycotoxin produced by a wide range of fungal species of the *Penicillium, Aspergillus*, and *Byssochlamys* genera (*Frisvad*, 2018; *Vidal et al.,* 2019). Chemically, PAT belongs to a group of compounds commonly known as toxic lactones (4-hydroxy-4H-furo(3,2-c) pyran-2(6H)-one). PAT is regarded as the most dangerous mycotoxin in injured fruits stored under improper environmental conditions (post-harvest). PAT is commonly investigated in apples and apple-derived foods (*Torovic et al.,* 2017), and it has been reported that approximately 50% of the apple juice samples analyzed worldwide contained relatively high detectable PAT levels (*Ying et al.,* 2018). Moreover, organic apples have higher PAT contamination than do

**Table 3.** The incidence of mycotoxins in foodstuffs in Serbia (2012–2019)

| Mycotoxins | Commodity | Method of analysis | N (%) | Range (μg kg$^{-1}$) | Mean (μg kg$^{-1}$) | Ref. |
|---|---|---|---|---|---|---|
| AF-s | Maize | ELISA | 13/29 (44.83) | | 13.95 | *Krnjaja et al., 2013a* |
| | | | 16/29 (55.17) | | >40 | |
| | | | 12/12 (100) | 0.33–2.40 | 1.39 | *Krnjaja et al., 2013b* |
| | | | 137 (68.5%) | 1.01–86.1 | 36.3 | *Kos et al., 2013a* |
| | | | | Up to 560 | 33,21 | *Stefanovic, 2014* |
| | | | 20/20 (100) | 2.31–3.34 (harvested) | 2.77 | *Krnjaja et al., 2015* |
| | | | 20/20 (100) | 1.03–4.11 (stored) | 2.16 | |
| | | ELISA | | 1.98–7.01 | 1,33 | *Jaksic et al., 2015* |
| | | HPLC-FLD | 103/180 (57.2) | 1.3–91.4 | 12.7 | *Janic Hajnal et al., 2017* |
| | | ELISA | 5 | 2.28–4.31 | 3.22 | *Kos et al., 2017a* |
| | | | 5–72.3 | 1.0–111.2 | 3.1–37.4 | *Kos et al., 2018* |
| | | | (50–87.5) | 1.3–1.9 | | *Krnjaja et al., 2018* |
| | | | 36/37 (97.3) | 0–491.7 | 60.3 | *Obradovic et al., 2018* |
| | | | 12/90 (18.9) | 0–27.9 | 1.3 | |
| | Flours of various cereals | HPLC | 5.2 | 1.59–4.76 | 2,13 | *Torovic, 2018a* |
| | Maize flour | | 48.2 | max. 9.14 | 0.55 | |
| FUM | Wheat | ELISA | 35/41 (85.4) | 750–2465 | 882.7 | *Stepanic et al., 2011* |
| | Maize | ELISA | 29/29 (100) | | 3590.00 | *Krnjaja et al., 2013a* |
| | | | 12/12 (100) | 880–2950 | 1610.83 | *Krnjaja et al., 2013b* |
| | | | 90/90 (100) | 520–5800 | 1730 | *Kos et al., 2014* |
| | | LC-MS/MS | 2/10 (20) | 75–561 (organic) | | *Vukovic et al., 2014* |
| | | | 4/9 (44) | 10–230 (conventional) | | |
| | | ELISA | 20/20 (100) | 1519–9780 (harvested) | 3700.84 | *Krnjaja et al., 2015* |
| | | | 20/20 (100) | 760–35760 (stored) | 5976.50 | |
| | | HPLC−FLD | 88–98 | Up to 20340 | 672–2290 | *Jaksic et al., 2015* |
| | | ELISA | 74 | 540.1–5076 | 2750 | *Kos, 2017a* |
| | | ELISA | 29/37 (78.4) | 0–10790 | 1300 | *Obradovic et al., 2018* |
| | | | 30/90 (33.3) | 0–10860 | 2800 | |
| | | | 37 (75.7) | 830–10.790 | 1406 | *Udovicki et al., 2019* |
| | | | 90 (34.4) | 930–10.860 | 1905 | |
| | | | 33 (93.9) | 1050–3790 | 580 | |
| | | | 98 (100) | 890–34.480 | 4310 | |
| | Corn flours | HPLC-UV | 51/56 (96.4) | Max.1468.5 | 205.5 | *Torovic et al., 2018b* |
| | Corn flake | | 11/15 (73.3) | Max. 579.4 | 87.3 | |

| Mycotoxins | Commodity | Method of analysis | N (%) | Range (μg kg$^{-1}$) | Mean (μg kg$^{-1}$) | Ref. |
|---|---|---|---|---|---|---|
| TCT | Cereal | LC | 15 (68.2) | 68–19.520 | 537 | *Jajic et al., 2011* |
| | Wheat | ELISA | 30/41 (73.2) | 50–5000 | 1988.1 | *Stepanic et al., 2011* |
| | | | 37/41 (90.2) | 25–135.6 | 24.2 | |
| | Wheat flour | UHPLC | 13 (86.7) | 17.5–976 | 325 | *Skrbic et al., 2012* |
| | | | 4 (26.7) | 9.8–26.9 | 4.1 | |
| | Crop maize | ELISA | 26/50 (52.0%) | 25.3–200 | 154.1 | *Janic Hajnal et al., 2013* |
| | Maize | ELISA | 22/29 (75.86) | | 235 | *Krnjaja et al., 2013a* |
| | | | 12/12 (100) | 41–226 | 128.17 | *Krnjaja et al., 2013b* |
| | | | 48/90 | 25.09–209.0 | 50.93 | *Kos et al., 2014* |
| | | | 2/90 | 600.0–700.0 | 650.0 | |
| | | ELISA | 20/20 (100) | 42–238 (harvested) | 117.83 | *Krnjaja et al., 2015* |
| | | | 20/20 (100) | 380–10.684 | 2034.4 | |
| | | | 52 | 275.2–882.1 | 541 | *Kos et al., 2017a* |
| | | ELISA | 2.5 | 260.1- 1388 | 642.3 | *Kos et al., 2017b* |
| | | | 96.0 | 260.4–9050 | 3063.3 | |
| | | | 15.5 | 252.3–6280.0 | 921.1 | |
| | | HPLC | 221/245 (54.3) | | 1806 | *Jajic et al., 2017* |
| | | ELISA | (22.2–100) | 445–1977 | | *Krnjaja et al., 2018* |
| | White wheat flour | | 23/45 (51) | 99–440 | 142 | *Jaukovic et al., 2017* |
| | Corn flour | HPLC-UV | 24/56 (42,9) | Max. 931.8 | 101.3 | *Torovic et al., 2018b* |
| | Corn flake | | 6/15 (40) | Max. 878.6 | 255.1 | |
| Alternaria toxins, TeA | Wheat | HPLC | 63/92 (68.5%) | 0.75- 48.9 | 18.6 | *Janic Hajnal et al., 2015* |
| AOH | | | 11/92 (12.0% | 0.49–70.2 | 39.0 | |
| AME | | | 6/92 (6.5%) | 2.5- 2676 | 92.4 | |
| PAT | apple-based food | HPLC | 32/114 (28.1 | 1–8.3 | 3.5 | *Torovic, 2017* |
| ZEA | Wheat | ELISA | 37/41 (90.2) | 10–1000 | 442.6 | *Stepanic et al., 2011* |
| | Wheat flour | UHPLC | 5 (33.3) | 1.9–21.1 | 4.6 | *Škrbic et al., 2012* |
| | Maize | ELISA | 35.0 | 1.81−3.32 | 2.67 | *Jakšić et al., 2011* |
| | | | 12/12 (100) | 15.44–188.05 | 71.79 | *Krnjaja et al., 2013b* |
| | | ELISA | 15 | 35.6-183.5 | 83.3 | *Kos et al., 2017a* |
| | | | (88.89–100) | 16.82-26.97 | | *Krnjaja et al., 2018* |
| | Corn flour | HPLC | 37/56 (66,1) | max. 242.1 | 15 | *Torović et al., 2018b* |
| | Corn flake | | 13/15 (86,7) | max. 121.6 | 13.6 | |
| OTA | Flours of various cereals | HPLC | 7/58 (29.3) | 0.07–23.04 | 2.04 | *Torović et al., 2018a* |
| | Maize | | 21/56 (37.5) | Max. 7.16 | 1.21 | |
| | Pig kidney | HPLC | 14/95 (14.74) | 0.10–3.97 | 1.36 | *Polovinski Horvatovic et al., 2019* |
| | Maize | LC–MS/MS | 13 (25) | 2–318 | 53 | *Kos et al., 2020* |
| | | | 9(18) | 0.5–27 | 6 | |

conventional apples, leading to consequent risk, particularly for infants and preschool children (*Marin et al.,* 2013). During the fermentation of apple juice to cider, PAT is completely destroyed.

Clinical signs usually include gastrointestinal symptoms (nausea, vomiting, gastric ulcers, intestinal haemorrhages, and lesions in the duodenum) that are accompanied by kidney damage. Chronic symptoms include genotoxic, neurotoxic, immunosuppressive and teratogenic effects (*Vidal et al.,* 2019). Furthermore, at the cellular level, PAT has been reported to lead to cell (plasma) membrane rupture, inhibition of protein synthesis, and inhibition of DNA and RNA synthesis. Regarding its carcinogenicity to humans, the IARC included PAT in category 3, as not classifiable (*Saleh and Goktepe,* 2019). The JECFA established a provisional maximum TDI (PMTDI) for PAT of 0.4 μg $kg^{-1}$ bw $day^{-1}$ (*JECFA,* 1995).

*Emerging mycotoxins*

Emerging mycotoxins are defined as mycotoxins that are neither routinely determined, nor legislatively regulated (*Gruber-Dorninger et. al.,* 2016). However, the evidence of their incidence is rapidly increasing (*Gruber-Dorninger et. al.,* 2016). The most relevant and frequently occurring emerging mycotoxins are *Fusarium* toxins. *Fusarium* emerging mycotoxins include enniatins (ENNs), beauvericin (BEA), moniliformin (MON) and fusaproliferin (FUS). Carcinogenicity, immunotoxicity and neurotoxicity are the main toxicological effects of emerging mycotoxins (*Cimbalo et al.,* 2020). Emerging fusariotoxins have mostly been investigated in Mediterranean countries. Moreover, their presence has been reported recently in maize from Serbia (*Jajic et al*., 2019; *Janic Hajnal, et al*., 2020). The authors found that MON, BEA and FUS had the highest presence among the emerging mycotoxins and were present in maize from all the investigated regions in the country (*Jajic et al*., 2019; *Janic Hajnal, et al*., 2020).

Also, emerging mycotoxins include citreoviridin, gliotoxin, griseofulvin, mycophenolic acid, β-nitropropionic acid, kojic acid, tremorgenic mycotoxins (penitrems, janthitrems, lolitrems, and the paspalitrems), penicillic acid, viomellein, vioxantin, xanthomegnin and walleminols. Their carcinogenicity to humans is not classifiable by IARC. Due to the lack of research showing direct human and animal

**Table 4.** The incidence of AFM1 in milk and dairy products in Serbia (2012–2019)

| Mycotoxins | Commodity | Method of analysis | N (%) | Range (μg $kg^{-1}$) | Mean (μg $kg^{-1}$) | exceed EU MRL n (%) | Ref. |
|---|---|---|---|---|---|---|---|
| AFM1 | Pasteurized and sterilized milk | HPLC | 54/60 (90) | 0.003–0.104 | 0.026 | 6 (10.0) | *Torovic,* 2015 |
| | Raw cow milk | ELISA | 540/678 (79.7) | 0.026–>1 | 0.282 | 14 (21) | *Tomasevic et al.,* 2015 |
| | Milk products | | 322/184 (57) | | 0.268 | 122 (37.8) | |
| | Heat treated milk | | 438/317 (72.4) | 0.026–0.50 | 0.090 | 3 (3.3) | |
| | Raw cow milk | HPLC | 32/38 (84) | 0.006–0.864 | 0.273 | 24 (63,16) | *Polovinski--Horvatovic et al.,* 2016 |
| | Raw cow milk | ELISA | 503/1207 (41.7) | 0.026–0.263 | 0.037 | 353 (29.25%) | *Miocinovic et al.,* 2017 |
| | Dairy products | | 236/997 (23.7) | 0.026–0.320 | 0.019 | 42 (4.21%) | |
| | Raw cow milk | | 4078/ 5054 (80.7) | 0.005–1.26 | 0.071 | 1557 (30.1) | *Milicevic et al.,* 2017a |
| | Heat-treated milk | | 1117/1233 (90.6) | 0.005–0.280 | 0.035 | 214 (17.3) | |
| | Dairy products | | 61/501 (12) | 0.005–0.147 | 0.021 | 3 (0,6) | *Milicevic et al.,* 2017b |
| | Raw cow milk | | 12268/ 15178 (81) | 0.005–1.09 | 0.055–0.074 | 3608 (23.7) | *Milicevic et al.,* 2019 |

**Table 5.** The incidence of mycotoxins in feed samples in Serbia (2012–2019)

| Mycotoxins | Commodity | Method of analysis | N (%) | Range (μg kg$^{-1}$) | Mean (μg kg$^{-1}$) | Ref. |
|---|---|---|---|---|---|---|
| AFB1 | Maize | ELISA | 59/70 (84.3) | 2.17–88.85 | 18.15 | *Ljubojevic et al.,* 2013 |
| | Cattle feedstuffs | | 66/67 (98.5) | 0.3–8.8 | 1.6–7.9 | *Krnjaja et al.,* 2013c |
| | Chicken feed | | 12/14 (85.71) | 1.79–16.01 | 4.47 | *Krnjaja et al.,* 2019 |
| | Hen feed | | 16/16 (100) | 1.34–18.29 | 4.56 | |
| OTA | Maize | | 11/28 (39.3) | 5.03- 11.99 | 8.05 | *Ljubojevic et al.,* 2013 |
| | Chicken feed | | 100 | 19.04–51.30 | 34.40 | *Krnjaja et al.,* 2014 |
| | Hen feed | | 100 | 28.34–65.30 | 43.89 | |
| DON | Cattle feedstuffs | | 62–67 (92.5) | 2.0–1149 | 11–694.2 | *Krnjaja et al.,* 2013 |
| | Maize | | 7/28 (25) | 82.0–792 | 239 | *Ljubojevic et al.,* 2013 |
| T-2 | | | 11/29 (37.9) | 54.7–374.0 | 113.4 | |
| ZEA | | | 10/28 (35.7) | 25.84- 130 | 73.34 | |
| | Cattle feedstuffs | | 67/67 (100) | 29.2–2477.5 | 64.9–2477.5 | *Krnjaja et al.,* 2013 |
| FUMs | Pig feed | | 28/30 (83) | 218–3540 | 893 | *Jaksic et al.,* 2018 |
| | Horse feeds | HPLC | | 1680–6050 | 7,73 | Jovanovic *et al.,* 2016 |
| | Pig feed | | 3/3 (100) | 12–775 | | *Milicevic et al.,* 2016 |
| Mycophenolic acid | | | 3/3 (100) | 97–142 | | |
| DON | | | 3/3 (100) | 930–8220 | | |
| 3-DON | | | 1/3 (33) | <0.05–570 | | |
| 15-DON | | | 3/3 (100) | 58–451 | | |

health effects, there are no current regulations implemented regarding the presence of these emergent mycotoxins in food or feed.

The first preliminary study of the natural occurrence of *Alternaria* toxins (alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA)) in wheat from different wheat-growing areas from Vojvodina, Serbia, was conducted during 2011–2013. Among 92 analyzed wheat samples, 63 (68.5%) were contaminated with TeA, 11 (12.0%) with AOH and 6 (6.5%) with AME (*Janic Hajnal et al.,* 2015). This study also revealed the incidence of *Alternaria* toxin in wheat shows considerable variations from year to year, depending on weather conditions. Humid conditions (above 75% RH) and higher temperatures in the period from flowering to wheat harvesting could lead to increased fungal growth and mycotoxin production (*Janic Hajnal et al.,* 2015).

## Mycotoxin Exposure and Associated Human Health — Risk Assessment

A report from the Foodborne Diseases Burden Epidemiology Reference Group of the World Health Organization used global estimates of incidence to calculate illnesses, deaths, and disease attributable life years lost (DALYs), and revealed the highest global DALYs (636,869) were due to liver cancer attributed to aflatoxin (*Gibb et al.,* 2015). Among the four chemicals examined, aflatoxin was associated with the greatest number of DALYs. In the risk assessment context, chronic consumption of mycotoxin-contaminated foods has been linked to very broad effects, comprising essentially (*De Ruyck et al.,* 2015):

- carcinogenic effects, e.g., in lung, liver, kidney;
- genotoxic and/or mutagenic effects;
- toxic effects specifically in target organs such as kidney, liver, and the nervous and reproductive systems;
- teratogenic effects; and
- immunosuppressive effects.

Although mycotoxins have been clearly implicated in these health symptoms, many interacting factors in the pathogenesis of a mycotoxicosis make clinical signs and diagnosis complex and diverse. One of the most important health burdens associated with mycotoxin exposure is the development of cancers. In order to prevent cancer risks stemming from exposure to mycotoxins, the IARC has performed

the carcinogenic hazard assessment of some mycotoxins in humans, on the basis of epidemiological data, studies of cancer in experimental animals and mechanistic studies (*IARC,* 2015).

*Group 1 (Carcinogenic to humans)*

Among mycotoxicosis described in humans, aflatoxicosis is of the greatest concern. The IARC classified AFB1 in group 1, i.e., *carcinogenic to humans*. The symptoms of acute aflatoxicosis include oedema, haemorrhagic necrosis of the liver and profound lethargy (*Williams et al.,* 2004). Since 2004, multiple aflatoxicosis outbreaks have been reported worldwide, resulting in 500 acute illness and 200 deaths. Most of these outbreaks have been reported from rural areas and occurred because of consumption of home grown maize contaminated with moulds (*Wild et al.,* 2015). The occurrence of acute aflatoxicosis in humans after aflatoxin consumption has been reported in India, Malaysia and Kenya (*Richard,* 2007). Reports that evaluated outbreaks of aflatoxicosis have estimated acutely toxic and potentially lethal AFB1 doses in humans to be between 20 and 120 μg $kg^{-1}$ bw $day^{-1}$ when consumed over a period of 1 to 3 weeks (*Groopman et al.,* 2014).

Regarding chronic effects of aflatoxin exposure, almost 600,000 people globally die annually due to hepatocellular carcinoma (*Yang et al.,* 2019). This makes hepatocellular carcinoma the second most common cause of cancer deaths, following lung cancer, worldwide (*Dietrich et al.,* 2019). Of the more than 700,000 new hepatocellular carcinoma cases worldwide each year, about 25,200–155,000 can be attributable to aflatoxin exposure. Moreover, aflatoxin (when there is no involvement of Hepatitis B virus) could play a causative role in 4.6–28.2% of all global hepatocellular carcinoma cases (*Liu et al.,* 2012). A much larger public health concern is likely to be associated with the influence of mycotoxins in childhood health disorders. The associated mechanism, as previously discussed, involves metabolism of AFB1 in the liver to a highly reactive species capable of forming mutagenic DNA adducts. The association of Hepatitis B virus and aflatoxins with hepatocellular carcinoma is well established in less developed and developing countries (common in China and Africa), where 83% of new cases were registered in 2012 (*WHO,* 2015). A multinational study estimated the incidence of hepatocellular carcinoma could be decreased in high-risk areas by up to 23% if dietary aflatoxin exposure is thoroughly controlled and reduced below detectable levels (*Liu et al.,* 2012). Aflatoxins have been found in the tissues of children suffering from Kwashiorkor or Reye's syndromes (*Peraica et al.,* 2001), and the toxins were thought to be a contributing factor to these diseases. Reye's syndrome, which is characterized by encephalopathy and visceral deterioration, results in liver and kidney enlargement and cerebral oedema (*Wittenstein et al.,* 2004) Moreover, in children, aflatoxins cause reduced immunization efficiency that leads to enhanced risk of infections (*Kumar et al.,* 2017). Aflatoxins also have been reported to cause morphological changes in the testes, impaired sperm viability, poor foetal growth during pregnancy and stillbirth (*Smith et al.,* 2017).

In recent years in Serbia, most health concerns around mycotoxins have been related to aflatoxin exposure due to unusual contamination of maize and, consequently, of milk. A recent study conducted by *Udovicki et al.* (2019a) to asses aflatoxin exposure revealed a high average dietary intake (5.59 ng $kg^{-1}$ bw) through maize consumption in Serbia. Taking into account the contamination level of AFM1 in milk (*Milicevic et al.,* 2017), the highest estimated daily intake (EDI) values for AFM1 in raw milk were calculated for infants (1–4 years old) (2.257 and 2.206 ng $kg^{-1}$ bw $day^{-1}$ for males and females, respectively). The EDI values for AFM1 were found to decrease with increasing age; thus, the lowest values were recorded for adult females (age 16–25 years; 0.144 ng $kg^{-1}$ bw $day^{-1}$) and males (age >25 years; 0.168 ng $kg^{-1}$ bw $day^{-1}$). The estimated intakes of AFM1 in this study (*Milicevic et al.,* 2017) show lower levels of exposure of the Serbian adult population during 2015 and 2016 in comparison with the estimate of AFM1 intake reported in previous studies (*Torovic et al.,* 2015; Skrbic et al*.,* 2014; *Kos et al*., 2014). However, different study settings and methods were used, which do not allow results to be easily compared. Besides infants and young children, groups that are commonly recognized as populations vulnerable to AFM1 exposure, *Udovicki et al.* (2019b) identified the student population of Serbia as a group particularly vulnerable to AFM1 exposure in recent years, due to outbreaks of aflatoxin contamination. The model estimation was performed via probabilistic Monte Carlo simulation, deriving a mean AFM1 EDI for the student population in the range of 1.238 to 2.674 ng $kg^{-1}$ bw $day^{-1}$ (depending on the number of intake days considered). Similarly, EFSA has concluded that highest estimated chronic dietary exposure to AFB1 was in the young population groups, and the highest estimated chronic dietary exposure to AFM1 was in

infants and toddlers, which can be explained by their specific consumption patterns that are mostly based on milk and milk products (*EFSA*, 2019).

Biomarkers of exposure (in serum, urine and milk) are useful tools to complete the information about exposure, metabolism, toxicology and the carry-over rates of the different parent compounds or metabolites in the context of human (particularly infant) or animal exposure to mycotoxin hazards (*De Ruyck et al.,* 2020). Due to the long half-life of albumin in humans, the measurement of aflatoxin albumin adducts and their derivatives in blood are strongly preferred, and indicate an exposure extent over 1–2 months (*Leong et al.,* 2012).

*Group 2B (Possibly carcinogenic to humans)*

Group 2B applies to agents for which there is some evidence from human, experimental animals and/or mechanistic data that they can cause cancer in humans, but the data are still far from being conclusive. Consumption of FUM-contaminated foods by humans has been correlated with increased incidence of oesophageal cancer, hepatotoxicity (hepatocellular carcinoma), and nephrotoxicity in populations in various parts of the world (*Waskiewicz et al.,* 2012). Similar observations have been reported from China (*Xue et al.,* 2019), Italy (*Franceschi et al*., 1990) and Brazil (*Van Der Westhuizen et al.,* 2003). The ratio of sphinganine/sphingosine in serum, plasma or urine has been used as a biomarker of exposure to FUMs (*Solfrizzo et al.,* 2011). Recently, interest has emerged in the link between FUM exposure and child growth impairment. Based on the study by *Chen et al.* (2018) and several other studies, a link was found between FUM exposure and child growth impairment. The prevalences of child growth stunting are highest in sub-Saharan Africa, South Asia, and Central America. The incidence of neural tube defects was linked with high FUM intake by women along the Mexico-Texas border (*Voss and Riley,* 2013). However, in a second evaluation in 2002, there was inadequate evidence in humans for the carcinogenicity of FB1, which confirmed the classification of FB1 as possibly carcinogenic to humans (Group 2B) (*IARC,* 2002).

The kidney is considered to be the major target organ for OTA effects. OTA, which has been classified by the IARC as a group 2B carcinogen, is the most potent renal carcinogen in all mammalian species reported to date (*IARC,* 2014). OTA has been associated with human disorders, chronic interstitial nephropathy and Balkan endemic nephropathy in the former Yugoslavia, associated with urothelial cancer (*Pavlovic*, 2013). Human diets that exceed OTA levels of 70 μg $kg^{-1}$ $day^{-1}$ can result in renal tumours (*Reddy and Bhoola*, 2010). It can cross the placenta and accumulate in foetal tissue, causing various morphological anomalies. Early life exposure to OTA can cause testicular cancer in men (*Bayman and Baker*, 2006). Biomarkers for dietary exposure are reflected in OTA levels in plasma, serum and urine, and in breast milk particularly in an assessment of the risk for infants (*Valetta et al.,* 2018). Recently, *Zhang et al.* (2009) reported induction of apoptosis in neuronal cells that might be a contributing factor to the pathogenesis of neurodegenerative diseases like Alzheimer's and Parkinson's diseases. At present, new information regarding the genotoxicity of OTA (formation of OTA-DNA adducts), its role in oxidative stress and the identification of epigenetic factors involved in OTA carcinogenesis could lead to classification in Group 2B (Possibly carcinogenic to humans) (*Ostry et al.,* 2017).

*Group 3 (Not classifiable as to its carcinogenicity to humans)*

Other mycotoxins, i.e., PAT, citrinin (CIT), ZEA, TCTs (in particular T-2 toxin), NIV and DON, are considered by IARC as "*not classifiable as to its carcinogenicity to humans*" (Group 3). Group 3 applies to agents for which there are too limited, inadequate, or no data to allow classification. DON has been reported as the causative agent of Kashin-Beck disease (KBD), an endemic, chronic and deformed osteoarthropathic disease, which mostly occurs from north-eastern to south-western China, south-eastern Siberia and North Korea (*Li et al.,* 2016). Previously, it was reported that DON induced gastrointestinal poisoning and was a suspected aetiologic agent of gastroenteritis in children (*CDCP*, 1999). Prior to the discovery and implementation of reliable methods for the analysis of mycotoxins, *Fusarium* species were implicated in several human outbreaks of mycotoxicoses. Alimentary toxic aleukia in Russia from 1932 to 1947 was correlated with cereal grains contaminated with *Fusarium sporotrichioides* and *Fusarium poae*. Symptoms included mucous membrane hyperaemia, oesophageal pain, laryngitis, asphyxiation, gastroenteritis, and vertigo. With regard to the relatively recent studies considering risk assessment (EDI) of PAT intake through apple-based products by infants and children in Serbia, the results revealed no health risk for Serbian infants and preschool children through apple-based foods

(*Torovic et al.,* 2017). In contrast to the situation for aflatoxins (*Udovicki et al.,* 2019a; 2019b), in Serbia, DON and ZEA intake by adults was 0.262 and 0.05 μg $kg^{-1}$ bw $day^{-1}$, respectively, through consumption of wheat-based food. Only 3.96% and 2.25% of the population exceeded established TDI values for DON and ZEA, respectively (Djekic et al., 2019).

CIT, a secondary metabolite of *Penicillium citrinum*, has been associated with yellowed rice disease in Japan. Considering its co-occurrence with OTA, CIT is responsible for nephropathy in pigs and other animal species (*Rasic et al.,* 2015). According to a recent EFSA report, occurrence data are lacking for a correct risk assessment of citrinin. Associated health risks of ergot mycotoxins are not of much significance today, and human ergotism is extremely rare (*Peraica et al.,* 1999), probably due to two reasons: primarily, recent improvements in grain cleaning and milling processes that are able to remove most of the ergots, leaving very low levels of the alkaloids in the flour, and; secondly, these alkaloids might be relatively unstable and can be destroyed easily by conventional processing (baking, cooking, milling).

## Current methods in risk assessment of genotoxic and non-genotoxic compounds

Additional to the principles described by the *IARC* (2015), methodologies for human and animal risk assessments have been improved further, with a special emphasis on animal exposure assessment. EFSA guidance includes new approaches to risk assessment, such as the use of the Margin of Exposure (MOE). The MOE is a tool used by risk assessors to characterize the risk from exposure to genotoxic and carcinogenic substances that can be found in food or feed. The MOE is the ratio between the benchmark dose level (BMDL) that causes a 10% increase in cancer incidence in animals ($BMDL_{10}$) and the total intake ($MOE=BMDL_{10}/EDI$). For substances that are both genotoxic and carcinogenic, the EFSA Scientific Committee (*EFSA*, 2012) stated that an MOE of 10,000 or higher, if based on a $BMDL_{10}$ from an animal carcinogenicity study, would be of low health concern. However, it provides an indication of the level of safety concern about a substance's presence in food but does not quantify the risk. The EFSA CONTAM Panel concluded that calculated $BMD_{10}$ values through carcinogenicity data in animal experiments were 170 ng $kg^{-1}$ bw per $day^{-1}$ and 21.0 μg $kg^{-1}$ bw $day^{-1}$ for AFB1 and OTA, respectively (*EFSA,* 2006; *EFSA,* 2007).

In the case of non-genotoxic compounds, EFSA have determined hazardous quotients for the TDI and TWI of several food contaminants. TDI is used for food contaminants, while acceptable daily intake (ADI) is used for food additives. For the purpose of risk characterization, the hazard index (HI) should be used. The HI is the ratio between EDI and TDI. A value higher than 1 indicates a risk for consumers. Table 1 includes TDI and PTWI for the major mycotoxins previously described.

### *Cancer incidence in Serbia*

Chronic non-communicable diseases, comprising cardiovascular diseases, malignant tumours, diabetes, obstructive lung disease, injury and poisoning, mental health disorders and other chronic diseases have dominated Serbia's national disease pathology for decades. In fact, they constitute the major contributor to the burden of disease in terms of disability-adjusted life years (DALYs) or mortality. The cause of death for almost one in five persons who died (21.3%) was malignant tumour. Cancer incidence data in Serbia are collected and reported by the Institute of Public Health of Serbia (2019). According to data from Cancer Registry of Central Serbia, in 2015, 27,867 new cases of malignant tumours (14,582 men and 13285 women) were registered, and 15,224 people (8,790 males and 6,434 females) died of cancer. Furthermore, men were mostly diagnosed with and died of bronchus and lung cancer, colon and rectum cancer, and prostate cancer. In women, the most frequent sites of malignant tumours were breast, colon and rectum, and bronchus and lung.

The cancer incidence rate in males was 297.6 per 100,000 population, and in females 256.7 per 100,000 population. The highest cancer rates in males were registered in the City of Belgrade (349.7 per 100,000) and in the District of Pirot (347.0 per 100,000) and in females in the City of Belgrade (301.3 per 100,000) and in the District of Sumadija (295.3 per 100,000). The possible mechanisms of the genotoxicity of AFB1 and AFM1 and associated co-factors that act synergistically (both high and/or low doses) such as other mycotoxins, hepatitis viruses B and C and cyanotoxins, and their roles as hepatocarcinogens are still unclear in this Serbian case study. Considering the increasing number of malignant tumours in our country, further research into the relationship between the occurrence of mycotoxins and cancer incidence is required.

## Impact of mycotoxins on animal health and productivity

In animals, mycotoxins produce a broad range of harmful effects to livestock health, production and welfare (*Pleadin,* 2015). The main effects include reduction in animal productivity, increased incidence of disease due to immuno-suppression, damage to vital organs accompanied by pathological change, interference with reproductive performance, little or no response to veterinary therapy, and in some extreme cases, death (*Yang et al.,* 2020). Because of their co-occurrence usually at low concentrations, mycotoxins can cause subclinical losses in production and increase the risk and incidence of other diseases. One of the first indications of a chronic mycotoxicosis is growth depression, which can result from reduced feed intake, impaired nutrient utilization and changes in feed quality or toxicity (*Bryden,* 2012). Among the animals, poultry, pigs and aquatic vertebrates are very sensitive to mycotoxins (*Oliveira and Vasconcelos*, 2020).

In animal studies, poultry are reported as the most sensitive domestic animals to aflatoxin toxicity. Ducks are the most sensitive to aflatoxins followed by turkeys, quail, broilers and layers. Aflatoxin toxicity in poultry causes fatty liver and kidney disorders, leading to numerous illnesses. OTA has been linked with porcine nephropathy in the Balkans. Lower concentrations of OTA in pigs are of major concern due to the mycotoxin's distinct toxicokinetic characteristics including long plasma elimination half-life and entero-hepatic and renal recirculation tissue accumulation (*Milicevic et al.,* 2008). Poultry are generally less prone to the effects of OTA due to birds excreting OTA faster than mammals, leading to more limited accumulation. The elimination half-life of OTA in broiler chickens is significantly shorter than that in pigs (4 h versus 150 h, respectively), leading to a lower systemic exposure of OTA in chickens *(Duarte et al.,* 2011). Differences in sensitivity between avian species are as follows: duck s>broiler>chickens>turkeys>Japanese quail ($LD_{50}$ values for birds are 0.5, 3.3, 5.9 and 16.5 mg $kg^{-1}$ $bw^{-1}$, respectively) (*Puvaca et al.,* 2012). Otherwise, turkeys seem to be more susceptible to several other mycotoxins than ducks, broiler chickens, or laying hens. These differences in sensitivity between avian species can be attributed to differences in toxicokinetics of the mycotoxin (*Guerre*, 2015). Impairment of feather cover could result in the carcass being downgraded due to blemishes and scratches on exposed skin. It has also been demonstrated that skin pigmentation is reduced during aflatoxicosis and ochratoxicosis, probably due to decreased absorption of dietary carotenoids.

Among animals, in almost all the species tested, FB1 has been shown to be hepatotoxic and nephrotoxic. In orally exposed animals, FUMs are in general poorly bioavailable, rapidly distributed mainly to liver and kidney, extensively biotransformed and rapidly excreted, mostly *via* the faecal route. Despite the fact that the toxicity of FUM is low, it has been linked with several diseases in domestic animals: equine leukoencephalomalacia (ELEM) in horses, recently recorded in Serbia (*Jovanovic et al.,* 2015), and porcine pulmonary oedema syndrome (PPE) in swine. PPE is observed in animals exposed to low levels of FUM (3–10 mg FB1 $kg^{1}$ feed) (*Souto et al.,* 2015). Evaluations of outbreaks of ELEM in the USA showed that consumption of feed containing more than 10 mg FB1 $kg^{-1}$ feed was associated with increased risk of ELEM, while no increased risk was found for feed containing less than 6 mg $kg^{-1}$ feed (*Ross et al.,* 1990). The potential for FUM contamination in animal food products such as milk and eggs is of concern due to their widespread consumption and, especially for milk, the exposure potential of children (*Voss et al.,* 2007).

In domestic animals, ZEA poisoning has been associated with hyperoestrogenic or feminizing syndromes. Pigs are generally the most affected animal, in which it causes genital/urinary problems (*Zielonka et al.,* 2020). The major symptoms of ZEA poisoning include hyperaemia and oedematous swelling of the vulva in prepubertal gilts and in severe cases, prolapse of the vagina and rectum. In pregnant gilts and sows, ZEA can increase abortion, stillbirths and neonatal mortality. In male pigs, atrophy of the testes occurs with decreased libido and hypertrophy of the mammary glands (*Danicke et al.,* 2015). Poultry are the least affected of the livestock animals after ingestion of ZEA. Zeranol, a derivative of ZEA, is used in some countries as a growth promoter for sheep and cattle.

The clinical symptoms of TCT toxicosis vary from acute mortality to reduced growth and productivity. Group A TCTs (T-2 and HT-2 toxins) are of major concern as they are more toxic than the type B TCTs (deoxynivalenol and NIV). Group A TCTs induce necrotic changes in the mouth and gastrointestinal tract, emesis, diarrhoea, anorexia, haematological and immunological alterations and sometimes even a lethal outcome. Group B TCTs (at concentrations of 2–5 mg $kg^{-1}$) are associated with feed refusal, and concentrations > 20 mg $kg^{-1}$ will induce

emesis, especially in pigs, the species most susceptible to this mycotoxin (*Haschek et al*., 2013). Consumption of low levels of these mycotoxins, especially in combination with the stress of commercial production, can result in chronic effects including impaired immunity and decreased resistance to infectious diseases. Consumption of vomitoxin-contaminated products has been correlated with reduced milk production in dairy cattle, vomiting in swine, and inhibition of reproductive performance and immune function in several animal species. T-2 toxin is rapidly metabolized and eliminated in different animal species, and therefore, there is no evidence for tissue accumulation or transfer into milk (*Li et al.,* 2011). In an outbreak of T-2 toxicosis, the rate of egg production decreased from 51% to 72%, while the incidence of cracked eggs increased from 3% to 15%. These clinical signs were accompanied by thinner and more fragile shells and would also have implications for hatchability.

Key information derived from animal exposure assessment to mycotoxins includes the amount and nature of residues of the parent compound and/or its biologically active metabolites, which can occur in animal-derived products, such as milk, meat and eggs. Thus, the prediction of potential residues and/or metabolites of contaminants is an important objective of human exposure assessment. There are significant differences among pig and poultry tissue deposition studies due to differences in absorption and metabolism of toxins and their metabolites. In general, residues of the mycotoxins ZEA, TCTs and FUMs are not considered to be of public health importance, as only very low levels of the toxins were found in the tissues of animals that had been fed very high levels of the toxins in experimental situations (*Fink-Gremmels et al.,* 2019).

## Major techniques for the determination of mycotoxins in Serbia

For the purposes of risk assessment, confirming the diagnosis of a mycotoxicosis, and for monitoring mycotoxin mitigation strategies, it is important to use sensitive, specific, and reproducible methods for mycotoxin analysis in various food matrices. Mycotoxin analysis in food and feed is generally a multistep process comprised of sampling, sample preparation, toxin extraction from the matrix (usually with mixtures of water and polar organic solvents), extract clean-up and finally detection, and quantitative determination. A successful detection method should be robust, selective, sensitive and flexible regarding the expandability to other mycotoxins. Methods commonly used for mycotoxin detection and quantification in Serbia are presented in Tables 3–5. Besides conventional laboratory methods such as enzyme-linked immunosorbent assay (ELISA) and chromatographic methods (high performance liquid chromatography (HPLC), liquid

**Table 6.** Advantages and disadvantages of different mycotoxin analysis methods commonly used in Serbia

| Method | Advantages | Disadvantages |
|---|---|---|
| HPLC | Good sensitivity, selectivity and repeatability, automated, short analysis times, official method of mycotoxin analysis. | Expensive equipment and analysis costs, requires trained and skilled personnel, destructive sample preparation, may require derivatization, time consuming, laboratory use only. |
| LC-MS | Multi-mycotoxin analysis, low LOD/LOQ, good sensitivity, selectivity and repeatability no derivatization required, automated, gold standard of mycotoxin analysis. | Very expensive equipment and analysis costs, requires highly trained personnel, sensitivity relies on ionization, matrix assisted calibration curve due to matrix interferences, time consuming, laboratory use only. |
| ELISA | Fast, relatively easy to use, simple sample preparation, inexpensive equipment, low limit of detection, simultaneous analysis of multiple samples, limited use of organic solvents, possible automatization, screening method. | High level of cross reactivity with related mycotoxins, possible false positives, matrix interference problems, narrow detection range, semiquantitative, laboratory use only. |
| LFIA | Fast, no clean-up, inexpensive equipment, easy to use, no specific training required, screening method, on-site analysis. | High level of cross-reactivity with related mycotoxins, validation required for additional matrices, semiquantitative. |

chromatography coupled with mass spectrometry (LC-MS)), in recent years, several lateral flow immunoassays (LFIA) have become available on the Serbian market, providing the possibility of on-site mycotoxin screening. Major advantages and disadvantages of the mycotoxin analysis methods usually used in Serbia are presented in Table 6.

New developments in mycotoxin analysis focus on faster, multi-mycotoxin, environmentally friendly, cost-effective and fit-for-purpose methods in food, feed, biological tissue and body fluids. Today, the food industry clearly has a need for both rapid screening techniques, which could be also used outside the laboratory environment, and high sensitivity-precision methods for confirmatory purposes. Novel materials, methods and techniques for this purpose are developed daily. Among novel materials aptamers, molecular-imprinted polymers (MIPs) and various nano materials (nano metals, quantum dots etc.) have great potential for use in mycotoxin analysis. Sometimes termed chemical antibodies, aptamers are single-stranded oligonucleotides of DNA or RNA sequences (usually 25–80 bases long) that are produced by an *in vitro* selection process called systematic evolution of ligands by exponential enrichment (SELEX) and have high affinity and specificity target molecules. To date, several techniques for sample clean-up (based on SPE/IAC technology) and mycotoxin analyses have been developed (*Yang et al.,* 2013). MIPs, a robust alternative to natural recognition elements (antibodies and biological receptors), have also found use in sample clean-up and mycotoxin analysis (*Mueller and Appell,* 2016). Current trends in food analysis are focused on application of fast, easy to use, and cheap biosensor technologies (surface plasmon resonance, surface-enhanced Raman scattering, piezoelectric, fluorescence polarization) that are able to detect with high sensitivity and selectivity various compounds connected with food quality and safety (*Puiu et al.,* 2014; *Evtugyn et al.,* 2017). Following the success story of glucose biosensors, and with the use of novel materials, the development of biosensors for mycotoxin detection and quantification provides the perspective for cost-effective, small portable devices allowing precise and high-throughput on-site measurements; these should prove to be valuable tools in protecting human health. Concerning confirmation methods, there is a clear trend towards the use of multiple-analyte methods, mostly based on ultrahigh-performance liquid chromatography (UHPLC) coupled with mass spectrometry, with various mass analyzers allowing the use of streamlined sample preparation procedures that save time and labour and reduce the overall costs associated with mycotoxin testing (*Malachová et al.,* 2018).

Measuring equipment and measurement processes could produce incorrect results affecting the quality and validity of obtained results (*ISO*, 2013). Measurement errors and test uncertainties in the context of product (in this case product tested for mycotoxins) conformity assessment, in particular, highlight the increasing interest and enhanced insight into decision-making gained when extending classical, purely statistical treatment of consumer and producer risks. Samples of product are checked against a specification, but even if the mean mycotoxin concentration is under the specified limit, there is still a finite probability that mycotoxin concentration in the batch actually lies outside the limit (*Pendril,* 2006). This occurs because of non-zero measurement uncertainty and when the mycotoxin concentration is relatively close to the specified limit (*Pendril,* 2006). As a result of a recent ballot, *ILAC* has published guidelines to advise on this issue (*ILAC,* 2019). This publication was extensively revised by the ILAC Accreditation and Laboratory Committees to provide guidance to laboratories, assessors, regulators and customers in the use of decision rules when issuing statements of conformity to specifications or standards as required in the 2017 edition of ISO/IEC 17025 (*ILAC,* 2019). Here, the role of the ISO 17025 (*ISO*, 2017) accreditation process in order to ensure the overall quality of laboratory work and also to enforce confidence in any results obtained must be emphasized. However, the European Union Reference Laboratory (EURL) for mycotoxins has, together with its partners from the national reference laboratories, continuously monitored and also evaluated the performance of analytical methods with the aim of ensuring a reliable measurement capacity in Europe.

## Mycotoxin legislation and regulations

Since it is impossible to fully eliminate the presence of undesirable substances and contaminants in food and feed, legislation and regulation are constantly evolving issues. Besides the adverse health impacts they have on both humans and animals, the presence of mycotoxins negatively influences food and feed trade. Thus, maximum concentrations of mycotoxins should be set at strict levels, which are reasonably achievable considering the risk analysis related to food consumption (Table 1). Risk analysis is a key discipline for reducing food-borne

**Table 7.** Maximum residue levels for mycotoxins in foodstuffs, according to the European Union and Serbian legislation

| Mycotoxins | Foodstuffs | Maximum levels (μg kg$^{-1}$) | | |
|---|---|---|---|---|
| | | EU[1–5], SRB[6,7] | | |
| | | B1 | Sum of *AFs* | M1 |
| AFB1[2] | Groundnuts (peanuts) and other oilseeds. Hazelnuts and Brazil nuts, to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs, with the exception of: — groundnuts (peanuts) and other oilseeds for crushing for refined vegetable oil production | 8.0 | 15.0 | |
| | Almonds, pistachios and apricot kernels to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs | 12.0 | 15.0 | |
| | Tree nuts. Dried fruit. Maize and rice to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs. | 5.0 | 10.0 | |
| | Groundnuts (peanuts) and other oilseeds and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs, with the exception of: crude vegetable oils destined for refining, refined vegetable oils.<br>Tree nuts, dried fruit and processed products thereof intended for direct human consumption or use as an ingredient in foodstuffs.<br>All cereals and all products derived from cereals, including processed cereal products. | 2.0 | 4.0 | |
| | Almonds, pistachios and apricot kernels, intended for direct human consumption or use as an ingredient in foodstuffs | 8.0 | 10.0 | |
| | Hazelnuts and Brazil nuts, intended for direct human consumption or use as an ingredient in foodstuffs. | 5.0 | 10.0 | |
| | Raw milk, heat-treated milk and milk for the manufacture of milk-based products | | | 0.05/ 0.25[6] |
| | Spices: | 5.0 | 10.0 | |
| | Processed cereal-based foods and baby foods for infants and young children | 0.10 | | 0.10[7] |
| | Dietary foods for special medical purposes intended specifically for infants | | | |
| | Infant formulae and follow-on formulae, including infant milk and follow-on milk | | | |
| OTA[1] | Unprocessed cereals Roasted coffee beans and ground roasted coffee, excluding soluble coffee | 5.0 | | |
| | All products derived from unprocessed cereals, including processed cereal products and cereals intended for direct human consumption | 3.0 | | |
| | Dried vine fruit (currants, raisins and sultanas), Soluble coffee (instant coffee) | 10.0 | | |
| | Wine, grape juice, concentrated grape juice as reconstituted, grape nectar | 2.0 | | |
| | Processed cereal-based foods and baby foods for infants and young children. Dietary foods for special medical purposes intended specifically for infants | 0.50 | | |
| PT[1] | Fruit juices, concentrated fruit juices as reconstituted and fruit nectars. Spirit drinks, cider and other fermented drinks derived from<br>apples or containing apple juice | 50.0 | | |
| | Solid apple products, including apple compote, apple puree intended for direct consumption | 25.0 | | |
| | Apple juice and solid apple products, including apple compote and apple puree, baby foods other than processed cereal-based foods for infants and young children | 10.0 | | |

| Mycotoxins | Foodstuffs | Maximum levels (µg kg$^{-1}$) | | |
|---|---|---|---|---|
| | | EU[1–5], SRB[6,7] | | |
| | | B1 | Sum of *AFs* | M1 |
| DON[1] | Unprocessed cereals other than durum wheat, oats and maize | 1250 | | |
| | Unprocessed durum wheat, oats and maize | 1750 | | |
| | Cereals intended for direct human consumption, cereal flour (including maize flour, maize meal and maize grits), bran as end product marketed for direct human consumption and germ. Pasta (dry) | 750 | | |
| | Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals | 500 | | |
| | Processed cereal-based foods and baby foods for infants and young children | 200 | | |
| ZEN[1] | Unprocessed cereals other than maize | 100 | | |
| | Unprocessed maize. Maize intended for direct human consumption, maize flour, maize meal, maize grits, maize germ and refined maize oil | 200 | | |
| | Cereals intended for direct human consumption, cereal flour, bran as end product marketed for direct human consumption and germ. | 75 | | |
| | Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize snacks and maize based breakfast cereals. Maize snacks and maize based breakfast cereals | 50 | | |
| | Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children.<br>Processed maize-based foods for infants and young children. | 20 | | |
| FUM-s (B1+B2)[1] | Unprocessed maize | 2000 | | 4000 |
| | Maize flour, maize meal, maize grits, maize germ and refined maize oil | 1000 | | 1400–2000 |
| | Maize based foods for direct human consumption | 400 | | 800–1000 |
| | Processed maize-based foods and baby foods for infants and young children | 200 | | 200 |
| T-2+HT-2[3] | Unprocessed cereals | | | |
| | ▪ barley (including malting barley) and maize | 200 | | – |
| | ▪ oats (with husk) | 1000 | | – |
| | ▪ wheat, rye and other cereals | 100 | | – |
| | Cereal grains and products for direct human consumption | | | |
| | Oats. Oat bran and flaked oats | 200 | | – |
| | Maize. Cereal bran except oat bran, oat milling products other than oat bran and flaked oats, and maize milling products | 100 | | – |
| | Other cereals and other cereal milling products. | 50 | | – |
| | Breakfast cereals including formed cereal flakes | 75 | | – |
| | Bread (including small bakery wares), pastries, biscuits, cereal snacks, pasta | 25 | | – |
| | Cereal-based foods for infants and young children | 15 | | – |
| Ergot sclerotia and ergot alkaloids[4] | Unprocessed cereals (18) with the exception of corn and rice | 0.5 g kg$^{-1}$ | | 0.5 g kg$^{-1}$ |
| Citrinin[5] | Food supplements based on rice fermented with red yeast *Monascus purpureus* | 100 | | |

**Legend:** [1]-EC 1881/2006, [2]-EC165/2010; [3] EC 165/2013, [4]-EC 2015/1940; [5]-EC 2019/1901: [6]-Serbian Regulation 22/2018; 81/2019), 0.05 µg kg$^{-1}$ (since 1 December 2020), Total AFs (sum of AFB1, AFB2, AFG1, and AFG2). [7]- Serbian Regulation (7/2017)

**Table 8.** Maximum permitted content and recommendations for mycotoxins in feedstuffs according to EU and Serbian legislation (mg kg$^{-1}$)[1]

| Mycotoxins | Products intended for animal feed | Maximum content (mg kg$^{-1}$) | |
|---|---|---|---|
| | | EU[2–4] | SRB[5] |
| AFB1[2] | All feed materials | 0.02 | 0.03 |
| | Complete feedingstuffs for cattle, sheep and goats with the exception of: | | 0.02 |
| | ▪ complete feedingstuffs for dairy animals | 0.005 | 0.005 |
| | ▪ complete feedingstuffs for calves and lambs | 0.01 | 0.005 |
| | Complete feedingstuffs for pigs and poultry (except young animals) | 0.02 | 0.02 |
| | Other complete feedingstuffs | 0.01 | 0.01 |
| | Complementary feedingstuffs for cattle, sheep, goats, pigs and poultry (except complementary feedingstuffs for dairy animals, calves, lambs and young animals) | 0.02 | 0.02 |
| | Other complementary feedingstuffs | 0.005 | 0.01 |
| OTA[3] | Feed materials: cereals and cereal products | 0.25 | 0.25 |
| | Complete and complementary feedstuffs | | |
| | ▪ For pigs | 0.05 | 0.1 |
| | ▪ For poultry | 0.1 | 0.2 |
| DON[3] | Feed materials: | | |
| | cereals and cereal products, with the exception of maize by-products | 8 | 8 |
| | maize by-products | 12 | 12 |
| | Complementary and complete feedstuffs | 5 | 5 |
| | with the exception of: | | |
| | ▪ Complementary and complete feedstuffs for pigs | 0.9 | 0.9 |
| | ▪ Complementary and complete feedstuffs for calves (< 4 months), lambs and kids | 2 | 2 |
| FB1, FB2[3] | Feed materials: maize and maize by-products | 60 | – |
| | Complementary and complete feedstuffs for: | | – |
| | ▪ pigs, horses (Equidae), rabbits and pet animals | 5 | – |
| | ▪ fish | 10 | – |
| | ▪ poultry, calves (< 4 months), lambs and kids | 20 | – |
| | ▪ adults ruminants (> 4 months) and mink | 50 | – |
| ZEN[3] | Feed materials: | | |
| | ▪ cereals and cereal products, with the exception of maize by-products | 2 | 4 |
| | Maize by-products | 3 | 6 |
| | Complementary and complete feedstuffs: | | |
| | ▪ for piglets and gilts (young sows) | 0.1 | 0.2 |
| | ▪ for sows and fattening pigs | 0.25 | 0.5 |
| | ▪ for dairy cattle, sheep (including lambs) and goats (including kids) | 0.5 | 1.0 |
| T-2, HT-2[4] | Cereal products for feed and compound feed: | | – |
| | oat milling products | 0.25 | – |
| | other cereal products | 0.5 | – |
| | compound feed (with the exception of feed for cats) | 2 | – |
| Rye ergot | All feedingstuffs containing unground cereals | 1000 | 1000 |

**Legend:** [1]-Relative to a feedingstuff with a moisture content of 12%. [2]-Directive, 2002/32/EC, amended by Directive, 2003/100. [3]- 2006/576/EU (for DON, ZEN, FBs and OTA); [4]–2013/165/EU (for T-2 and HT-2). [5]- Serbian Regulation (39/2016).

illness and strengthening food safety systems based on scientific opinion. Factors influencing mycotoxin regulations include availability of toxicity data, availability of data on the occurrence in different commodities, survey analytical data, methods of sampling and analysis, and trade contacts with other countries (*van Egmond et al*., 2007). However, factors fundamental to a country's ability to protect its population from mycotoxins include the political will to address mycotoxin exposure and capability of testing food for contamination, which determines whether the requirements can be enforced.

In Serbia, in accordance with European Union regulation, maximum levels (MLs) are prescribed for 11 mycotoxins in food: AFB1 and AFM1 individually as well as the sum of aflatoxins (AFB1, B2, G1, and G2), FUMs (FB1, FB), OTA, patulin, DON, ZEA and ergot sclerotia (*Serbian Regulation,* 2019) (Table 7). Surprisingly, in Serbia the regulatory authorities have not established MLs for FUMs in feed, despite their widespread occurrence and their health hazards for animals (*Serbian Regulation*, 2016) (Table 8). An increasing number of residue studies suggest that EFSA's guidance for mycotoxin MRLs in feed and, consequently, residues in animal-derived products, results in food that presents minimal risk to human consumers (*Dongping et al.,* 2019). In order to ensure the safety of food, existing legislation in Serbia encourages producers and researchers to pay serious attention to food and feed production processes and to develop comprehensive quality policies and management systems to improve food safety. Monitoring and control systems as integral components of the food safety system has been established to obtain reliable information about the real exposure of human populations to mycotoxins and any risk for public health. The Ministry of Agriculture Forestry and Water Management has overall responsibly for food and feed monitoring. The Veterinary Directorate has been assigned overall responsibility for monitoring and controlling mycotoxins in animal-derived products, while the Plant Protection Directorate is responsible for the implementation of monitoring/official controls of mycotoxins in plants. The presence of selected mycotoxins (AFM1 in milk and OTA residues in kidney and liver of slaughtered animals) has been systematically monitored according to an annually planned monitoring program. Types, numbers of samples, and combinations of analyzed mycotoxins/groups of mycotoxins are planned annually, taking into account the number of slaughtered animals in previous years. The combinations of analyzed mycotoxins and matrices are chosen predominantly according to the Council Directive 96/23/EC. In addition, a monitoring program is conducted by the Institute of Meat Hygiene and Technology, Belgrade. Also, the official control of food and feed in Serbia covers samples from border inspection, samples that have been the subject of complaints or are derived from food/feed poisoning cases, and samples from any follow-up actions.

## Integrated food safety management system/ risk management and control strategies

Several codes of practice, including the Code for the Prevention and Reduction of Mycotoxins in Cereal Grains and Grain-Derived Foods and Feeds, have been developed by Codex Alimentarius. These recommendations are divided into two parts: pre-harvest practices based on good agricultural practice (GAP) and post-harvest practices such as good manufacturing practice (GMP) and good hygiene practice (GHP) that are implemented in hazard analysis and critical control point (HACCP) systems. An organization dealing with feed production and/or grain storage will develop a formal food safety management system (FSMS) to ensure that feed it produces is safe for consumption. Organizations need to establish and implement suitable control measures that are appropriate for the specific hazards existing in the food/feed and the risk they pose to the final consumer. The Codex standard (*CAC,* 2003a) for HACCP uses a decision tree in order to determine whether the hazard should be controlled as a CCP or not. It does not attempt to assist the organizations in determining what type of control should be employed where "Not a CCP" is the outcome. This makes it limited for most modern food businesses seeking to develop a robust food safety plan (*Politis et al.,* 2017). Storage conditions are one of the critical stages for the post-harvest prevention of mycotoxins. Among many factors within a storage ecosystem, temperature and humidity are crucial for the fungal infection and mycotoxin contamination. Maintaining uniform grain temperatures throughout the grain mass is important to avoid moisture imbalance. This can be achieved by passing large volumes of ambient air (aeration) through the grain mass. Improved storage management (GMP, GHP), especially at the farmer and small trader levels, will prevent fungal growth and mycotoxin contamination in stockpiled grains (*Milicevic et al.,* 2019b). However, GAP associated with prediction models that integrate the most important field parameters and weather inputs are the best options to prevent fungal colonization

and mycotoxin production in the field. If mycotoxin has occurred, contaminated feed and food must be managed through post-harvest decontamination/ detoxifying procedures to convert mycotoxins into non- or less toxic products (Figure 1).

Traditional detoxifying methods include physical, chemical and biological methods (*Wan et al.*, 2020). Mycotoxin decontamination by physical methods includes various procedures such as sorting and separation, immersing and washing, irradiation, filtering and adsorption. Novel processing technologies like microwave heating, gamma and electron beam irradiation, ultraviolet and pulsed light, electrolyzed water and cold plasma are also being continuously investigated. These practices, despite their various efficiencies, advantages and limitations, are applicable both in food and feed contaminated by various mycotoxins. Chemical treatments for mycotoxin decontamination involve bases, oxidizing agents, organic acids and other agents. Currently, application of chemical treatments for mycotoxin reduction in food or feed has many limitations due to consumers' health concerns. Also, losses in the nutritional value and the palatability of feeds and interactions with food components are disadvantageous factors of these methods (*Kolosova and Stroka*, 2011). Moreover, the use of chemical decontamination processes is not legal within the EU (*Directive,* 2002/32). Among them, only ammonia and ozone have been developed and utilized industrially. The main advantage of biological degradation of mycotoxins is that it works under mild, environmentally friendly conditions. Some microorganisms and/or enzymes can degrade mycotoxins into less toxic or non-toxic derivatives by transforming their toxicological properties. A wide range of bacteria (lactic acid bacteria), moulds and yeasts (*Saccharomyces cerevisiae*) have shown the ability to biodegrade mycotoxins. Moulds such as *Aspergillus, Rhizopus* and *Penicillium spp*. show effective abilities to detoxify mycotoxins (*Cheng et al.,* 2016). Recent research in Serbia indicates the biocontrol agent, a natively atoxigenic *A. flavus* strain, has high potential for reducing aflatoxin contamination in local environmental conditions (*Savic et al.,* 2020).

In 2009, the European Union approved the use of mycotoxin-detoxifying agents, by including a new group of feed additives defined as 'substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their

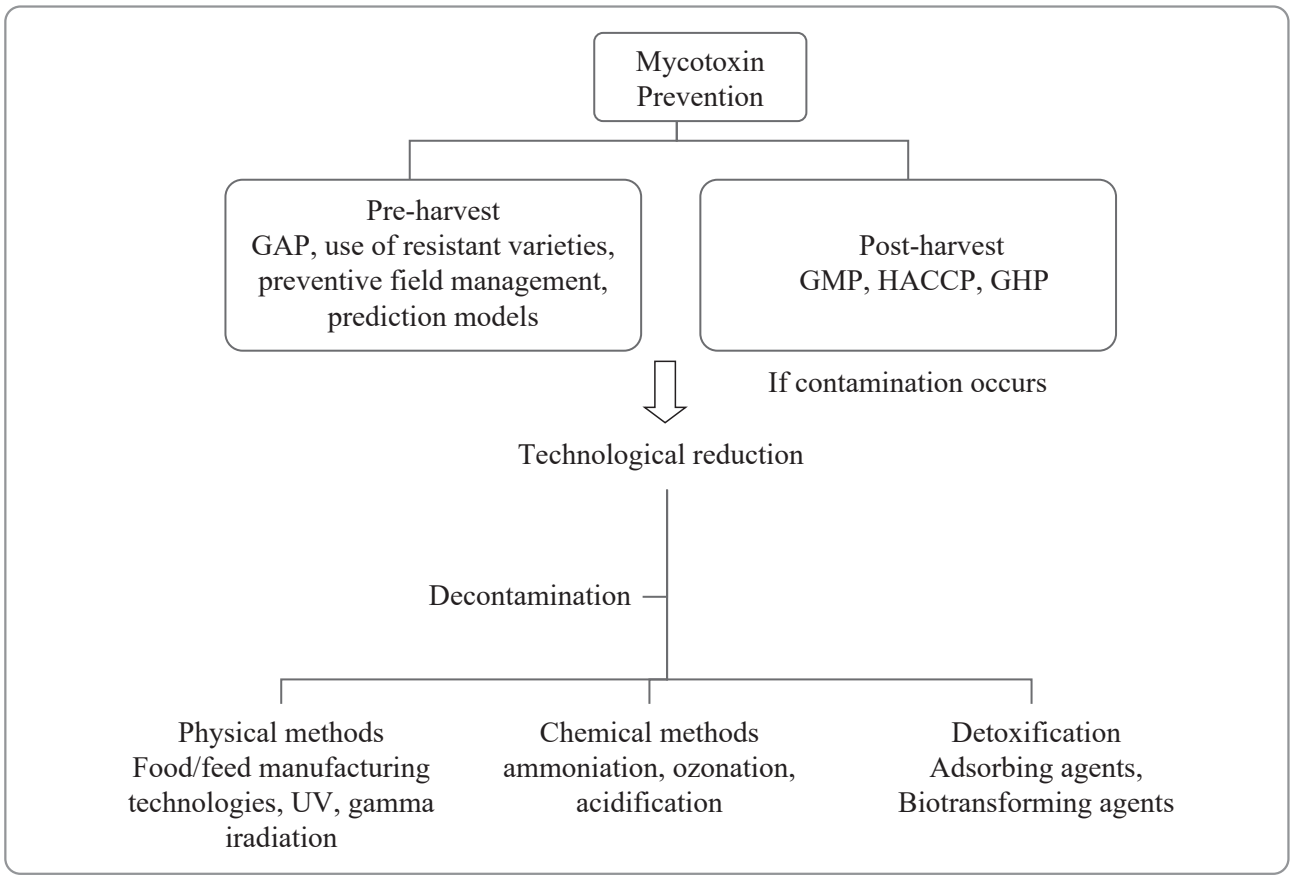


**Figure 1.** Strategies to prevent and reduce mycotoxins

**Table 9.** SWOT analysis for improving the national food safety system

| Strengths | Weaknesses |
|---|---|
| ▪ Harmonized national and EU legislation exist in the agri-food sector.<br>▪ Updated food laws, unified standards and conformity of assessment systems have all been addressed.<br>▪ National food contaminant monitoring programs to ensure prevention of potential risks are in place.<br>▪ The Consumer Protection Department has been established. The National Expert Council for Food Safety Risk Assessment has been established.<br>▪ Testing laboratories in the field of food safety are equipped with fit-for-purpose equipment, running validated methods and operating according to the requirements of ISO 17025. Food safety management is on the political agenda.<br>▪ The national action plan for public health highlights the necessity to perform research on food quality and safety.<br>▪ Availability of competent authorities to set national norms and standards.<br>▪ Food safety is based on food business operator responsibility.<br>▪ Mandatory HACCP/equivalent quality assurance system for producers. | ▪ Dual authority in policy making.<br>▪ Risk assessment has not yet been implemented. In a number of cases, inspection is carried out only after a problem arises. Lack of effective coordinated effort and flow of information between all of the authorities involved in the food control system (One Health).<br>▪ No food quality and safety control agency has been established.<br>▪ Absence of local risk (assessment) data. Inadequate data collection, storage and analysis.<br>▪ Lack of a national database on population characteristics of diet and food composition. The National Reference Laboratory is not operational.<br>▪ The Rapid Alert and Alert System (RASFF) has not been developed and implemented. |
| **Opportunities** | **Threats** |
| ▪ Adequate research infrastructures (RIs) in food, nutrition, and health domain are essential for nutrition epidemiology, innovative nutritional research, dietary exposure and food safety risk assessments and effective public health nutrition (PHN) strategies to address the diet-related diseases, malnutrition and foodborne diseases.<br>▪ Policymakers should be informed by timely, quality information on the severity of hazards, economic costs and the nutritional impacts, particularly in vulnerable groups.<br>▪ Harmonized and standardized food consumption data should be collected from national dietary surveys at individual level.<br>▪ The complex nature of food safety means a holistic and multidisciplinary approach needs to be developed.<br>▪ A regional mycotoxin risk assessment center must be established.<br>▪ The use of IoT, artificial and business intelligence, cloud systems, sensors and algorithms for generation, storage, interpretation and distribution of all relevant data for myctoxin management all require expert attention.<br>▪ Climate change aslo requires the development of predictive models to forecast and prevent mycotoxin contamination.<br>▪ Model developed in Serbia can be further disseminated and applied in other countries in the Balkans. | ▪ Food imports are steadily increasing, predominantly from at-risk regions. A large number of laboratories carrying out food analysis are not at the appropriate technical and technological level of equipment.<br>▪ Lack of: risk analysis capabilities in regulatory bodies; governmental budget allocated for policy implementation; knowledge within professionals and policy makers; adequate research infrastructure for food nutrition and health (FNH-RI).<br>▪ Low purchase power of consumers does not encourage operators to invest into costlier quality measures.<br>▪ Price of food commodities is the major factor in the consumer decision.<br>▪ Impoverished consumers are not protected from eating commodities rejected by exporters due to high contamination levels.<br>▪ Limited number of inspectors and limited scope in monitoring programs.<br>▪ Insufficient technological readiness for climate change. |

mode of action' (*EC*, 2009). These feed additives are known as detoxifying agents and were designed to reduce the potential adverse effects of mycotoxins after the feed is ingested by animals. These binding agents are roughly classified as 'inorganic' or 'organic'. The main mycotoxin-restricting mechanisms involved with these additives include: 1) physically binding the mycotoxins and, thus, decreasing the gastrointestinal absorption of mycotoxins and their distribution to blood and target organs; 2) inactivating mycotoxins, and; 3) degrading or transforming mycotoxins into less toxic metabolites (biotransformation) (*Peng et al.,* 2018). These feed additives also have some advantages and disadvantages. Due to their low costs and high efficacy, mycotoxin binders have been widely used by local farmers to reduce the potential adverse effects of mycotoxins. The main disadvantage is limited multi-toxin-binding efficacy, meaning even if a parent mycotoxin is deactivated, its metabolic products are not necessarily eliminated. With both low costs and low efficacy, these mycotoxin binders are sometimes added into feed in large amounts by farmers, which can decrease the total nutritional values of feed and result in nutritional imbalance in the animals (*Karlovsky*, 2011).

In Serbia, future *in vivo* research should include assaying naturally multi-contaminated feeds, which reflect real mycotoxin concentrations, taking into account EU-regulations and EFSA report endpoints. The use of mycotoxin-detoxifying feed additives, regarding their efficacy, safety and their potential for interactions with critical nutrients (vitamins and minerals) requires further study.

GHP and HACCP are the primary tools available to control chemical hazards in food operations. The basic idea of HACCP system is to manage food safety based on risk management principles and cover a range of biological, chemical and physical hazards (*Akkerman et al.,* 2010; *Maldonado-Siman et al.*, 2014). Historical and current thinking limits the scope of FSMS to the control and management of the aforementioned hazards, but does not included the wider consideration of prevention of NCDs, although it can be argued that NCDs could involve "conditions of food with the potential to cause an adverse health effect" (*Manning et al.,* 2019). It could be supposed that organizations need to consider how these developments will influence the categorization of food hazards and intoxication in the future (*Manning*, 2019) and the impact on management approaches to mycotoxin hazard control and management.

Recently, the food safety management approach has been completed and developed through the inclusion of other metrics like the Food Safety Objective (FSO) (*García-Cela et al.,* 2010). An FSO is defined in FSMSs as the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection (*CAC,* 2003b). In practice, FSOs are achieved through the establishment and implementation of performance and process criteria (performance criterion (PC), process criterion (PcC) and product criterion (PdC)). In every step of the food chain, it is necessary to know the effect of every treatment ensure hazard levels never overtake safety levels before the time of consumption (performance objective (PO)).

## Current and future outlook

Although the Serbian Food Safety Act is proactive and is based on CAC standards and on the principles of European Union legislation, there are some deficiencies in its development, implementation, control of the implementation and efficiency evaluation. These are required in order to further improve the country's food safety system. *FAO/WHO* (2003) documents, based on extensive experience in different systems, provide useful suggestions on how to effectively establish and maintain a food safety system. The use of key structural components of the FAO/WHO Guide (*FAO/WHO*, 2006) enables the identification of significant indicators and parameters of a food safety system, such as strength, weakness, potentialities and hazards, termed SWOT analysis (strengths, weaknesses, opportunities and threats). Using SWOT analysis as a basis, it is possible to make recommendations for improving the national food safety system (*Gurinovic*, 2016; *Gurinovic et al.,* 2018) (Table 9).

# Aktuelna situacija kontaminacije hrane i hrane za životinje mikotoksinima sa osvrtom na javnozdravstveni rizik u Srbiji

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*A p s t r a k t: Mikotoksini predstavljaju hemijski hazard mikorbiološkog porekla, proizvod sekundarnog metabolizma pretežno filamentoznih plesni. Značaj mikotoksina najčešće se vezuje za pojavu brojnih oboljenja kod ljudi i životinja, koja pripadaju grupi nezaraznih bolesti (eng. non-communicable diseases). Nezarazne bolesti, (npr. maligni tumori), vodeći su uzroci obolevanja, invalidnosti i prevremenog umiranja (pre 65. godine života) u svetu, a i u našoj sredini (eng. Disability Adjusted Life Years-DALY). Maligna oboljenja se karakterišu dužim vremenom trajanja i nastaju kao posledica interakcije mnogobrojnih faktora kao što su genetski, fiziološki status organizma, prirodno okruženje i biološkog odgovora čovekovog organizma. Sve veće interesovanje za sinergistički efekat sintetskih i prirodnih kontaminenata na zdravlje ljudi, ukazuje na to da kontaminacija mikotoksinima predstavlja idalje oblast od prioritetnog značaja za sve učesnike u lancu hrane. Uzimajući u obzir da kontaminacija hrane mikotoksinima prvenstveno zavisi od klimatskih faktora, ekstremne klimatske pojave kao što su suša i poplave poslednjih godina zabeležene u Srbiji, potvrđuju činjenicu da su mikotoksini jedan od hazarda u lancu hrane na koji klimatske promene imaju najveći uticaj. U ovom radu pokušali smo da analiziramo ključne faktore od značaja za kontaminaciju mikotoksinima, kao i da se ukaže na najnovije trendove i strategije u prevenciji štetnih efekata mikotoksina u lancu hrane, sagledavajući stanje i mogućnosti u Srbiji.*

***Ključne reči:*** *mikotoksini, zastuljenost, javno zdravlje, SWOT-analiza.*

**Disclosure statement:** No potential conflict of interest was reported by authors.

**Acknowledgment:** This study was supported by the projects no. TR 31008, and contract no. 453–03–68/2020–14/200050, both funded by the Ministry of Education, Science, and Technological Development of the Republic of Serbia.

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Paper received: March 9th 2020.

Paper corrected: April 1st 2020.

Paper accepted: March 19th 2020.

**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 637.5'64.043*
*ID: 16223241*
*https://doi.org/10.18485/meattech.2020.61.1.2*

*Original scientific paper*

# Effect of rearing system on carcass properties, chemical content and fatty acid composition of backfat from Mangalitsa pigs

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*A b s t r a c t: This research examined the effects of two rearing systems (conventional versus free-range) on carcass characteristics, and cholesterol content, chemical and fatty acid properties of the backfat from Mangalitsa pigs. Depending on the rearing system utilized and live weight observed, we found important differences in the heaviness of the cold and warm Mangalitsa carcasses. The maximum total cholesterol in the backfat of pigs reared outdoors was 46.96 mg kg$^{-1}$, while the maximum total cholesterol in backfat of conventionally-raised Mangalitsa pigs was 55.80 mg kg$^{-1}$. The backfat from free-ranging Mangalitsa pigs contained lower levels of PUFA n-6 and greater amounts of PUFA n-3. The ratio of PUFA/SFA was remarkably different in pigs raised in the two systems, whereas the ratio of MUFA/SFA was lower in the pigs reared outdoors. Based on these results, the selection of rearing system could affect the chemical properties and carcass characteristics of Mangalitsa backfat.*

***Keywords:*** *rearing system; indigenous breed; carcass traits; backfat; cholesterol; fatty acids.*

## Introduction

The Mangalitsa, a fatty type of pig, is an autochthonous swine breed in Serbia, where it has been present for more than 100 years. Today, breeding Mangalitsa pigs are commercialized by processing the high quality meat into products to attract growing interest in the food production and consumer markets. The Mangalitsa pig's future is heavily dependent on whether products derived from it can be used effectively, and whether long-term markets can be secured. Today, consumers not only select meat products according to perceived eating quality and accessible pricing, but they also consider the nutritional value and the ethical meat quality, as well as animal welfare issues and the level of impact on the environment caused by the production system. Another reason for choosing ecologically, non-intensively produced meat is the opinion that the flavour and nutritive value of this type of meat are superior as compared to meat grown in the conventional way (*Mapiye et al*., 2011, *Parunovic et al*., 2012a). The aim of this study was to explore differences between carcass properties, chemical and fatty acid composition and the cholesterol content in backfat of free-range and conventionally reared Mangalitsa pigs.

## Materials and methods

Twenty-four castrated male Mangalitsa pigs were selected from a herd in a breeding programme. Twelve of the Mangalitsa pigs were raised conventionally — six pigs per pen, allowing 6 m$^2$ living space for each animal. These uniform pens were part of a group inside a pig farmer's shed, which was enclosed by walls and covered with a roof. The airflow was controlled manually by opening or closing the windows. The floor of the pens was concrete, and one third had concrete slats above a faeces and urine drainage channel. The other 12 Mangalitsa pigs were allowed to range freely over an area of 8 000 m$^2$, and so had regular access to fresh grass pasture and fallen acorns.

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After reaching a live weight of 60 kg, both groups of pigs were fed a conventional slaughter-pig feeding mixture that was distributed *ad libitum*. The composition of this compound feed is given in Table 1.

**Table 1.** Feed ingredient composition and estimated analyses of the diet

| **Ingredients** [a] | **(% as-fed)** |
|---|---|
| Maize | 70.0 |
| Meal [b] | 14.0 |
| Soybean meal [c] | 9.0 |
| Sunflower meal | 4.0 |
| Chalk | 1.0 |
| Dicalcium phosphate | 1.0 |
| Sodium chloride | 0.5 |
| Lysine | 0.15 |
| Methionine and Threonine supplement | 0.15 |
| Total | 100.00 |
| *Estimated nutrient content [d] (%)* | |
| Crude protein (N × 6.25) | 13.00 |
| Crude fat | 3.62 |
| Crude dietary fibre | 3.87 |
| Crude ash | 4.88 |
| ME content, MJ $kg^{-1}$ | 13.05 |

**Legend:** [a] Ingredient composition (% as-fed); [b] (wheat feed flour, barley, wheat, oats, dehydrated lucerne flour); [c] (soya press cake, soya protein concentrate with fish oil) [d] Estimated analyses (%)

At the end of the trial, at live weights of between 80 kg and 120 kg, the Mangalitsa pigs were transported in the morning to the nearby commercial abattoir in Jagodina (approximately 8 km) in groups of a maximum of 6 animals. Pig groups were not mixed during transport. The animals were slaughtered at similar live weights (100 kg), but not similar ages, because this enabled slaughter and transport procedures to be standardized; differences in carcass weights among animals of similar ages would have overruled other effects on carcass conformation or meat quality.

The body weight of the trial pigs was measured prior to and shortly after slaughter. Carcasses contained heads, trotters and kidney fat. Each carcass was weighed warm and then chilled (4°C for 24 h). After 24 h of cooling at 4°C, backfat measurements were taken with a ruler above the *m. gluteus medius* at the carcass split-line, at these positions: at the beginning (P1); at the highest spot of the *m. gluteus medius* (P2), and; at the end of the muscle (P3). Carcass length was measured from the cranial edge of the *symphisis pubis* to the anterior edge of the atlas vertebrae. During routine carcass splitting and cutting, samples of the backfat were taken between the 13th and 15th thoracic vertebrae. Prior to laboratory analysis, all the samples were vacuum packed and kept frozen at approximately −20°C.

Chemical composition was determined by following the methods defined by the AOAC (*Association of Official Analytical Chemists*, 2016). Cholesterol concentration was determined by HPLC/PDA, on a HPLC Waters 2695 separation module, with Waters 2996 photodiode array detector. In order to determine the concentration of fatty acids, total lipids were extracted by a rapid extraction method, using solvents on the Dionex ASE 200. A homogenized sample, mixed with diatomaceous earth, was extracted with a mixture of hexane and isopropanol (60:40 v/v) in a 33 mL extraction cell at 100ºC and under nitrogen pressure of 10.3 MPa. The extract thus obtained was steamed in a nitrogen flow at 50°C until dry fat remains were obtained (*Spiric et al*., 2010). Fatty acids as methyl esters were detected by capillary gas chromatography with a flame ionization detector. A predetermined quantity of lipid extracts, obtained by the rapid extraction method, was dissolved in tert-butyl methyl ether. Fatty acids were converted to fatty acids methyl esters (FAME) with trimethylsulfonium hydroxide, according to the SRPS EN ISO 5509:2007 method. FAMEs were analysed with a GC-FID Shimadzu 2010 device (Kyoto, Japan) on a cyanopropyl-aryl column HP-88 (column length 100, internal diameter 0.25 mm, film thickness 0.20 μm). The injected volume was 1 μL. Temperatures of the injector and detector were 250ºC and 280ºC, respectively. Nitrogen was used as a carrier gas, 1.33 mL $min^{-1}$, with a split ratio of 1:50, while hydrogen and air were used as detector gases. The temperature of the column furnace was programmed to range between 120ºC and 230ºC. The total duration of analysis was 50.5 min. Methyl esters of acids were identified according to their retention times, which were compared with those of the mixture of methyl esters of fatty acids in the standard Supelco 37 Component FAME mix (*Spiric et al*., 2010).

Data was statistically analysed by the least squares method and the GLM procedure of the SAS 9.1.3 program package (SAS Inst. Inc. 2002–2003). Tukey's test was used to compare the mean values of the genotypes when they were significantly

different. Least squares of means (LSM) with respective standard errors of means (SEM) and significance levels are shown in the tables.

## Results and discussion

Table 2 shows the live weights and carcass properties of Mangalitsa pigs reared in the free-range and conventional systems. The final live weight of Mangalitsa pigs and rearing system had a strong effect ($P < 0.001$) on the warm and cold carcasses weights. Simultaneously, conventionally reared pigs tended to be heavier. In their study, *Petrovic et al*. (2010) found differences in average masses of warm and cooled carcass sides between breeds.

In the current study, lower dressing percentages of the warm and cold carcasses were found in the free-range reared Mangalitsa pigs than in those fed conventionally ($P < 0.001$). However, the final live weight had a notable effect ($P < 0.01$) on carcass dressing percentage (Table 2). *Hoffman et al*. (2003), in their study, detailed warm (77.5%–77.7%) and cold (75.9%–76.4%) dressing percentages of pig carcasses. This is explained by the difference being possibly because the intake of grass fibre led to a better developed digestive system (mainly the large intestine) (*Hoffman et al*., 2003). In our study, the rearing system had no impact on the body length of the pigs, whereas live weight did ($P < 0.001$; Table 2). Differences in cooler shrink were not noted between the Mangalitsa pigs reared in the two rearing systems. *Anupam et al*. (2010) analysed the slaughter performance of Ghungroo, a native swine breed, and found that they had cooler shrink values of between 1.90% to 5.48%. In the current study, backfat thickness measurements at three control points was higher in conventional pigs (P1 = 3.73 mm, P2 =X3.05 mm, P3 = 3.90 mm) ($P < 0.001$); however, this was explained by the live weight of the pigs at slaughter and not by the effect of rearing system.

Our research showed that free-range reared system had no effect on accumulation of subcutaneous fat (Table 2).

Comparisons of the means for the chemical composition of the backfat derived from the free-range and conventionally reared Mangalitsa pigs are presented in Table 3. Significant differences ($P < 0.001$) were observed in the backfat water content depending on rearing system. Free-range reared Mangalitsa pig backfat contained a greater percentage of water than the backfat of the conventionally reared pigs. The percentage of water in backfat of Mangalitsa pigs kept in the conventional rearing system was 2.43% lower than in the outdoor pigs. The established value of water content in backfat of free-range reared Mangalitsa pigs (6.96%) was similar to the values of 6.53% (Cinta Senese pigs), 6.9% (Large White) and 7.76% (crossbreed pigs) found

**Table 2.** Comparison of the least squares mean ± (SEM) for the slaughter traits of free-range and conventionally reared Mangalitsa pigs

| Trait | Rearing system | | Significance of the influence [a] | |
|---|---|---|---|---|
| | Conv.[b] (n[c] = 12) | FR[d] (n = 12) | RS | LW |
| Starting live weight 70 day age (kg) | 12.02 ± 1.02 | 12.18 ± 1.14 | * | / |
| Average slaughter age (days) | 397.57 ± 10.75 | 451.77 ± 10.75 | *** | / |
| Live weight (kg) | 102.58 ± 3.85 | 98.33 ± 3.37 | NS | / |
| Warm carcass weight (kg) | 79.72 ± 0.41 | 76.41 ± 0.41 | *** | *** |
| Cold carcass weight (kg) | 77.82 ± 0.44 | 74.23 ± 0.44 | *** | *** |
| Warm carcass DP[e] (%) | 78.94 ± 0.51 | 75.91 ± 0.51 | *** | ** |
| Cold carcass DP[e] (%) | 77.31 ± 0.45 | 73.69 ± 0.45 | *** | ** |
| Cooler shrink (%) | 2.40 ± 0.22 | 2.85 ± 0.22 | NS | NS |
| Carcass length (cm) | 89.20 ± 0.61 | 89.22 ± 0.61 | NS | *** |
| Thickness of backfat $P_1$ (mm) | 61.70 ± 1.48 | 57.97 ± 1.48 | NS | *** |
| Thickness of backfat $P_2$ (mm) | 54.44 ± 1.91 | 51.39 ± 1.91 | NS | *** |
| Thickness of backfat $P_3$ (mm) | 59.95 ± 1.83 | 56.05 ± 1.83 | NS | *** |

**Legend:** [a] Significance level for rearing system (RS) and live weight (LW); [b] Conv. - Conventionally reared pigs; [c] n - number of samples; [d] FR - Free-range reared pigs; [e] DP: dressing percentage; $P_1$ - sacral point 1; $P_2$ - sacral point 2; $P_3$ - sacral point 3; NS — not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

by *Franci et al*. (2005). Pugliese *et al*. (2005) found 6.53% water in backfat of free-range Cinta Senese pigs and 5.34% in pigs kept in a conventional rearing system. In our study, the differences in main protein values between the two groups were significant ($P < 0.001$). Mangalitsa pigs reared conventionally had lower protein levels in the backfat than did free-range pigs. The live weight and the rearing system significantly influenced total fat content in the backfat (Table 3). In conventionally reared pigs, higher total fat content (3.27% higher) was determined than in the free-range group. The calculated fat/protein proportion was lower in backfat of the free-range reared Mangalitsa pigs compared with the conventionally reared group ($P < 0.001$).

In our study, the type of rearing system had an important effect on cholesterol content in the backfat of Mangalitsa pigs ($P < 0.05$; Table 4). The total cholesterol concentration of the backfat for pigs reared outdoors ranged between 31.40 mg $kg^{-1}$ to 46.96 mg $kg^{-1}$, while the cholesterol concentration of conventionally raised Mangalitsa pigs ranged between 37.35 mg $kg^{-1}$ to 55.80 mg $kg^{-1}$. *Csapó et al*. (2002) reported that the Mangalitsa pig fat had changeable cholesterol levels which ranged between 71 mg $kg^{-1}$ and 109 mg $kg^{-1}$. There was no truth in reports demonstrating that the fat of Mangalitsa pigs contains less cholesterol than that of other types of fattening pig (*Csapó et al*. 2002). *Kovács* (2009) noted that the average cholesterol content in *m. longissimus dorsi* of Mangalitsa pigs was 52 mg $kg^{-1}$.

Table 4 presents the fatty acid profiles of the backfat in the Mangalitsa pigs reared free-range and conventionally. In both rearing systems, palmitic acid (C16:0), oleic acid (C18:1 n-9) and linoleic acid (C18:2 n-6) were the most common SFA, MUFA and PUFA, respectively, in the backfat of the pigs. The backfat of outdoor-reared Mangalitsa pigs contained less PUFA than pigs fed conventionally and reared indoors. These variations were created mostly by higher total n-6 PUFA levels in the backfat of the conventionally reared Mangalitsa pigs ($p < 0.001$), and likewise by slightly higher levels of total n-3 PUFA ($P < 0.05$) in free-range reared pigs. These caused lower n-6/n-3 ratios in the backfat of the free-range reared Mangalitsa pigs feeding on acorns and grass pasture ($P < 0.01$) (Table 4). Consequently, in spite of the fact that the n-6/n-3 ratios in pigs in our study were always higher than dietary guidelines (*British Nutrition Foundation*, 1994), free-rearing seems to be a beneficial way to reduce this ratio in porcine animals.

Our investigation is similar to research by *Hansen et al*. (2006), who showed that organic pigs had lower MUFA and higher PUFA levels than conventionally-reared pigs. In our research, the higher C18:2 n-6 concentrations in free-range Mangalitsa pigs contributed to their total PUFA concentration ($8.27 \pm 0.22$) in comparison with that of the Mangalitsa pigs reared conventionally ($9.19 \pm 0.22$). Higher MUFA levels were found in outdoor-reared Iberian pigs and this compound was also detected in intramuscular fat (*Andrés et al*., 2001). Table 4 presents the higher MUFA/SFA and PUFA/SFA ratio we ascertained for conventionally-reared pigs in comparison with the free-range Mangalitsa pigs. In contrast, the total MUFA/PUFA ratio of the backfat was not different between the two groups of Mangalitsa pigs. Differences in fatty acid profile between conventional and free-range Mangalitsa pigs are likely a consequence of the different feeds. The fatty acid profile of the intramuscular fat is affected by various factors; generally, diet appears to be one of the most important factors. The PUFA/SFA ratio is the second index normally used to estimate the nutritional value of fats, and the recommended value for human dietary needs is 0.45 (*Department of Health*, 1994). For *m. semimembranosus* from Mangalitsa pigs, values

**Table 3.** Comparison of the least squares mean ± (SEM) for the chemical composition and fat/protein ratio of backfat of free-range and conventionally reared Mangalitsa pigs

| Item | Rearing system | | Significance of the influence [a] | |
|---|---|---|---|---|
| | Conv.[b] (n[c] = 12) | FR[d] (n = 12) | RS | LW |
| Water content (%) | 4.53 ± 0.25 | 6.96 ± 0.25 | *** | NS |
| Protein content (%) | 1.45 ± 0.10 | 2.30 ± 0.10 | *** | NS |
| Total fat content (%) | 94.02 ± 0.31 | 90.75 ± 0.31 | *** | * |
| Ash content (%) | 0.06 ± 0.01 | 0.08 ± 0.01 | NS | NS |
| Fat / protein ration e | 67.09 ± 2.79 | 41.04 ± 2.79 | *** | NS |

**Legend:** [a] Significance level for rearing system (RS) and live weight (LW); [b] Conv. - Conventionally reared pigs; [c] n - number of samples; [d] FR - Free-range reared pigs; [e] Fat / protein ratio was calculated. NS — not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

**Table 4**. Comparison of the least squares mean ± (SEM) for the fatty acid composition (%) and cholesterol content (mg kg$^{-1}$) of backfat from free-range and conventionally reared Mangalitsa pigs

| Item | Rearing system | | Significance of the influence [a] | |
|---|---|---|---|---|
| | Conv.[b] (n[c] = 12) | FR[d] (n = 12) | RS | LW |
| C14:0 | 1.10 ± 0.03 | 1.38 ± 0.03 | *** | NS |
| C16:0 | 24.60 ± 0.26 | 27.49 ± 0.26 | *** | NS |
| C16:1 | 2.79 ± 0.15 | 3.47 ± 0.15 | ** | NS |
| C17:0 | 0.36 ± 0.02 | 0.38 ± 0.02 | NS | NS |
| C17:1 | 0.22 ± 0.01 | 0.25 ± 0.01 | * | *** |
| C18:0 | 10.94 ± 0.29 | 12.67 ± 0.29 | *** | NS |
| C18:1 *cis*-9 | 49.64 ± 0.24 | 45.55 ± 0.24 | *** | NS |
| C18:1 *trans*-9 | 0.55 ± 0.03 | 0.56 ± 0.03 | NS | NS |
| C18:*1 cis*-11 | 4.81 ± 0.15 | 4.77 ± 0.15 | NS | NS |
| C18:2 cis n-6 | 8.22 ± 5.72 | 14.65 ± 5.72 | NS | NS |
| C18:3 *n*-3 | 0.19 ± 0.02 | 0.41 ± 0.02 | *** | NS |
| C18:3 *n*-6 | ND | ND | NS | NS |
| C20:0 | 0.21 ± 0.01 | 0.18 ± 0.01 | * | NS |
| C20:1 *n*-9 | 0.99 ± 0.03 | 1.07 ± 0.03 | NS | NS |
| C20:2 *n*-6 | 0.47 ± 0.06 | 0.56 ± 0.06 | NS | NS |
| C20:3 *n*-3 | 0.04 ± 0.00 | ND | *** | NS |
| C20:3 *n*-6 | 0.39 ± 0.03 | 0.43 ± 0.03 | NS | NS |
| C20:5 *n*-3 | ND | ND | NS | NS |
| C22:1+C 20:4 | 0.15 ± 0.01 | ND | *** | NS |
| C22:5 *n*-3 | ND | ND | NS | NS |
| C22:6 *n*-3 | ND | ND | NS | NS |
| SFA | 37.21 ± 0.36 | 42.11 ± 0.36 | *** | NS |
| MUFA | 59.14 ± 0.32 | 55.66 ± 0.32 | *** | NS |
| PUFA | 9.19 ± 0.22 | 8.27 ± 0.22 | ** | NS |
| USFA | 68.34 ± 0.44 | 63.93 ± 0.44 | *** | NS |
| Total *n*-3 PUFA | 0.37 ± 0.02 | 0.41 ± 0.02 | NS | NS |
| Total *n*-6 PUFA | 9.01 ± 0.21 | 7.87 ± 0.21 | *** | NS |
| MUFA/PUFA | 6.48 ± 0.15 | 6.76 ± 0.15 | NS | NS |
| MUFA/SFA | 1.59 ± 0.02 | 1.32 ± 0.02 | *** | NS |
| PUFA/SFA | 0.25 ± 0.01 | 0.20 ± 0.01 | *** | NS |
| USFA/SFA | 1.84 ± 0.03 | 1.52 ± 0.03 | *** | NS |
| *n*-6/*n*-3 PUFA | 25.23 ± 1.05 | 19.75 ± 1.05 | ** | NS |
| Cholesterol | 45.75 ± 1.61 | 40.14 ± 1.61 | * | NS |

**Legend:** [a] Significance level for rearing system (RS) and live weight (LW); [b] Conv. - Conventionally reared pigs; [c] n - number of samples; [d] FR - Free-range reared pigs; ND - not detected; SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, USFA - monounsaturated fatty acids + polyunsaturated fatty acids; Content of SFA, MUFA, PUFA — calculated from all detected acids; NS — not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

for PUFA = 14.94 and SFA = 32.43 (PUFA/SFA = 0.46), for *m. longisimuss dorsi* PUFA = 7.37, SFA = 36.58 (PUFA/SFA = 0.20) and for backfat PUFA = 13.90, SFA = 40.41 (PUFA/SFA = 0.34). Fats with low PUFA/SFA ratios are considered unfavourable, since they could cause increases in cholesterolaemia. Fat from, in particular, pasture-fed ruminants, normally contains PUFA/SFA in ratios below that recommended (*Sañudo et al*., 2000). *Nantapo et al*. (2014) examined the influence of genotype on fatty acid profiles of cow milk. They concluded that health-related ratios such as n-6/n-3 fatty acid ratios and PUFA/SFA ratios did not differ among bovine genotypes. However, indexes such as PUFA/SFA,

based only on the chemical structure of fatty acids, may not be adequate to estimate nutritional value of fats, because they take into account the preposition that all SFA cause an increase in cholesterol, but they ignore the effects of MUFA.

## Conclusions

Our study leads to the conclusion that free-range-reared Mangalitsa pigs had a lower backfat thickness than pigs reared in the conventional manner. In this study, the free-range rearing of Mangalitsa pigs produced higher protein, ash and water levels and lower total fat content and fat/protein ratios in the backfat, compared with conventionally housed and fed animals. Mangalitsa pigs reared in the conventional way produced backfat with higher MUFA, PUFA and USFA levels, higher concentrations of PUFA n-6, plus higher PUFA/SFA and n-6/n-3 ratios in the backfat in comparison with Mangalitsa pigs when reared free-range. The choice of rearing system had an important influence on cholesterol levels in Mangalitsa pig backfat. These potential applications to affect fatty acid composition of pork and products thereof could be of great interest, considering the increase in consumer concerns about food origin, safety and nutritional value. Accordingly, animal feeding outdoors, on pasture, appears to be an interesting strategy to improve the healthful image of (organic) pork from the human health point of view.

# Uticaj sistema gajenja na kvalitat trupa, hemijski sastav i sadržaj masnih kiselina leđne slanine Mangulica

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*A p s t r a k t: Cilj ovog rada bio je uticaj dva sistema gajenja (konvencionalni i slobodni uzgoj) na karakteristike trupa, kao i na sadržaj holesterola, hemijski sastav i sadržaj masnih kiselina leđne masnoće Mangulica. U zavisnosti od sistema gajenja i posmatrane telesne mase, utvrđene su značajne razlike u masi hladnih i toplih polutki Mangalica. Maksimalni ukupni holesterol u masnoći svinja koje se gajene u slobodnom sistemu uzgoja bio je 46,96 mg kg$^{-1}$, dok je maksimalni ukupni holesterol u masnoći konvencionalno uzgajanih Mangalica bio 55,80 mg kg$^{-1}$. Masnoća Mangalica koja je gajena u slobodnom sistemu imala je manji sadržaj PUFA n-6 i veći sadržaj PUFA n-3. Odnos PUFA / SFA bio je izuzetno različit kod svinja koje su gajane u dva sistema, dok je odnos MUFA / SFA bio manji kod svinja koje su gajane u slobodnom sistemu. Na osnovu ovih rezultata, izbor sistema za uzgoj svinja mogao bi da utiče na hemijska svojstva i karakteristike trupa Mangalica.*

***Ključne reči:*** *sistem gajenja; autohtona rasa; osobine trupa; leđna slanina; holesterol; masne kiseline.*

**Disclosure statement:** No potential conflict of interest was reported by the authors.

**Acknowledgments:** This research was part of the project (31081) "Application of different breeding, selection and biotechnological methods for refinement of pigs", funded by the Ministry of Education, Science and Technological Development, Republic of Serbia. The authors would like to thank all participants in the project and in this paper.

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Paper received: March, 27th 2020.

Paper accepted: April 9th 2020.

**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 637.5:631.563.9*
*ID: 16253193*
*https://doi.org/10.18485/meattech.2020.61.1.3*

*Original scientific paper*

# Changes in the physicochemical and microbiological properties of pork and chicken meats at ambient storage condition

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*A b s t r a c t: Pork and chicken meat samples were collected from pre-selected slaughterhouses to characterize the pH, titratable acidity (%TA) and aerobic plate count (APC) from slaughter until end of shelf-life at ambient temperature (30±2°C). Results showed that the population of microorganisms on meat samples increased over the storage time. On the other hand, pH and % TA were variable, showing no statistically significant changes throughout the storage period. Based on microbiological analysis, the shelf-life of pork and chicken meats ranged from 8 to 12 h and 3 to 6 h, respectively. Pearson correlation revealed there was no significant relationship between APC and pH of pork ($r = -0.10$, $n = 278$, $p > 0.05$) or between APC and %TA of pork ($r = 0.053$, $n = 278$, $p > 0.05$). On the other hand, there was a weak negative relationship between APC and pH in chicken ($r = -0.165$, $n = 267$, $p < 0.005$) and a positive relationship between APC and %TA ($r = 0.401$, $n = 266$, $p < 0.005$). This showed that pH cannot be used as a good indicator of meat spoilage. Furthermore, the differences between fresh and obviously spoiled meat samples, for both pH and %TA, were not great enough for practical use.*

***Keywords:*** *Pork, Chicken, pH, Aerobic Plate Count, Titratable Acidity*

## Introduction

Pork and chicken meats are among the most popular and widely consumed livestock meats all over the world (*FAO*, 2014). In the Philippines, pork meat had an average volume production of more than 2 billion kg annually in the period 2011–2016, which is the highest among all types of meat as recorded by the Philippine Statistics Authority (*PSA*, 2017). It was followed by chicken with an average volume production of more than 1.6 billion kg in the same period (*PSA*, 2017). Newly slaughtered meats such as pork and chicken are traditionally handled, distributed, and marketed in the Philippines at ambient temperatures in wet markets for a specified period of time within the day of slaughter (*National Meat Inspection Services*, 2012). According to *Tejada et al.* (2013), a lot of low-income Filipino consumers do not have refrigerators and rely only on wet markets for their daily freshly slaughtered meat supply, buying just in time for consumption on the same day.

Handling of fresh meat at ambient temperature is not widely accepted in some other countries. According to *Koutsoumanis et al.* (2006), temperature conditions higher than 10°C during transportation, retail storage and consumer handling can result in an unexpected loss of quality and a significant decrease of meat shelf-life. On the contrary, the local regulation reiterates that the traditional practice in the Philippines of handling and distributing of newly slaughtered meat has a historical record of safe consumption, having no public health problem traceable to the product (*NMIS*, 2012). As such, the Codex Code of Hygienic Practice for Meat (*CAC/RCO 58*, 2005), which recommends that meat be held at "temperatures that achieve safety and suitability objectives" is prescribed. We believe this recommendation is too non-specific, and thus could be open to several interpretations.

Temperature of meat during storage and initial microbial level are major factors affecting the shelf-life and quality of raw meat (*Koutsoumanis et al.*, 2006). Metabolic activities of microorganisms responsible for spoilage can produce metabolites that result in off-odours, greater exudate viscosity, and chemical modifications in the meat once the

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microbial population exceeds 7 log CFU $g^{-1}$ (*Raab et al.,* 2008). Since freshness of meat is the main criterion that influences the purchasing decision of consumers, it is imperative to determine the end of shelf-life and to assess the quality changes of meat during storage conditions without strict temperature control, as this is commonly practiced by our target population.

Shelf-life determination and assessment of quality changes of meat are commonly done by chemical, microbiological, and sensory methods or their combinations. Although the microbial method is the most desirable from the theoretical point of view, this technique requires two days or more for incubation and results (*Dainty*, 1996). Alternatively, the strong relationship between spoilage from the growth of bacteria and chemical indices could be used as a supplementary technique in determining end of shelf-life and assessing meat quality (*Dainty,* 1996). Some literature studies recognize pH and % titratable acidity (based on lactic acid) determinations as essential indicators of microbial spoilage in meat (*Nassos et al.,* 1983; *Hernández-Herrero et al.*, 1999). The practical advantage of using the pH determination lies in its simplicity and rapidity (*Pearson*, 1968). Thus, the objective of this study was to come up with a profile of the changes in the microbiological and physicochemical changes in pork and chicken meats exposed to ambient temperature (30±2°C). This study also aimed at investigating the relationships of the measured quality parameters in the hope of coming up with a chemical change-based indicator of microbial spoilage.

## Materials and Methods

### *Preparation of meat samples*

Pork loins, specifically the loin centre cut (approximately 5 kg), from newly slaughtered carcasses were obtained from a preselected slaughterhouse in Valenzuela City, Philippines. A total of eleven (11) pieces of pork loin was collected on six different sampling dates at 45 mins after slaughter. Each sampling date represents one (1) independent run while each piece of pork loin per run represents the internal replicate. Collected samples were packed in a sterile polyethylene (PE) bag, placed in a cooler box containing ice (1–4°C), and were immediately transported within 45 mins to the Food Processing Division (FPD) of Industrial Technology Development Institute (ITDI) to avoid further contamination. Upon arrival at FPD, the pork loins were deboned, and the skin and fats were trimmed under aseptic conditions. Pork loins were then cut into sample blocks of 3.0 cm × 3.0 cm × 2.0 cm (approximately 20 g), which were placed in sterile petri dishes. Meat samples were stored at ambient temperature (30±2°C) and were analysed every hour up to twelve hours.

Chicken breasts (approximately 500–600 g) were obtained from newly slaughtered broilers in a preselected poultry dressing plant in Valenzuela City, Philippines. About 3 kg, i.e., 6 pieces of chicken breast were collected randomly per run at 20 mins *post mortem*, and this was repeated on ten (10) different sampling dates. Collected chicken breasts were packed in sterile PE bags and were brought to the laboratory at FPD-ITDI in a cooler box containing ice. At the laboratory, chicken breasts were skinned and deboned. The resulting chicken breast fillets were then cut into sample blocks, stored, and monitored in a similar manner as pork loin samples.

### *Quality deterioration monitoring*

For the pH determinations, 5 g of meat sample was mixed with 45 ml of freshly boiled distilled water (cooled in a closed container at room temperature prior to use) using a stomacher (Lab-blender 80, Seward, England) for 1 minute. The supernate was used for pH determination. The pH was measured using a digital pH meter (LAQUA-PH1100, Horiba Scientific, Japan) at room temperature. Each value is the mean of three repeated measurements (*Zhang et al*., 2012).

The titratable acidity (TA, % lactic acid) was determined following the procedure described by *Shelef and Jay* (1970). Meat (10 g) was mixed with 200 ml of distilled water using a stomacher. The supernate was transferred into a 250 ml volumetric flask and distilled water was added until the volume reached the 250 ml mark. The supernate was then filtered through Whatman filter paper No.1. A 25 ml volume of filtrate was added to 75 ml distilled water with three drops of 1% phenolphthalein indicator solution and was titrated against 0.1 N NaOH endpoint, indicated by a faint pink colour which persisted for 30 seconds. TA was calculated using the equation given below:

$$\text{A, \% lactic acid} = \frac{\text{ml of 0.1 N NaOH} \times 0.1 \times \text{meq wt of lactic acid}}{\text{weight of sample (g)}} \times \times 100 \times \text{Dilution Factor (1/10)}$$

The aerobic plate count (APC) of meat samples was determined using 3M™ Petrifilm™. Briefly, a 10 g portion of meat sample was aseptically transferred to a sterile stomacher bag and mixed with

90 ml of 0.1% sterile peptone (HiMedia, India). The mixture was stirred for 2 minutes using a stomacher. The resulting supernate was serially diluted up to $10^7$ dilutions by transferring 10 ml of previous dilution to 90 ml of diluent for better enumeration. From the prepared serial dilutions, 1 ml of each dilution was pour plated in triplicate on APC petrifilms. The inoculated petrifilms were incubated at 35°C for 48 h (AOAC International, 2012). After incubation, colonies that emerged on the petrifilms were counted and interpreted using the interpretation guide provided by 3M™ Petrifilm™. APC were expressed as log CFU (colony-forming units) per gram of meat sample.

### *Statistical Analysis*

Descriptive statistical analysis using minimum, maximum, mean and standard deviation was utilized to describe the pH, APC, and %TA of meat. Pearson correlation coefficient was computed to describe the strength and direction of the relationship between the measured quality parameters at $p<0.05$. Data obtained in the study was also subjected to one-way analysis of variance (ANOVA) to determine if there were statistically significant differences among samples during storage at $p<0.05$. The Tukey's test was used as the *post hoc* test for samples showing significant differences. All Statistical analysis was computed using the IBM Statistical Packages for Social Sciences (SPSS) Statistics 22 (SPSS, Inc. 2013, New York, USA) software.

## Results and Discussion

### *Changes in pH, %TA, and APC in pork and chicken meats*

The changes in pH, %TA and APC of pork loin and chicken breast fillet throughout the storage at ambient temperature for twelve hours are shown in Figures 1 and 2, respectively. The initial mean pH of pork was 6.22±0.37 and %TA was 0.81±0.19%. For chicken meat, initial pH and %TA values were 6.15±0.22 and 0.82±0.07%, respectively. pH values

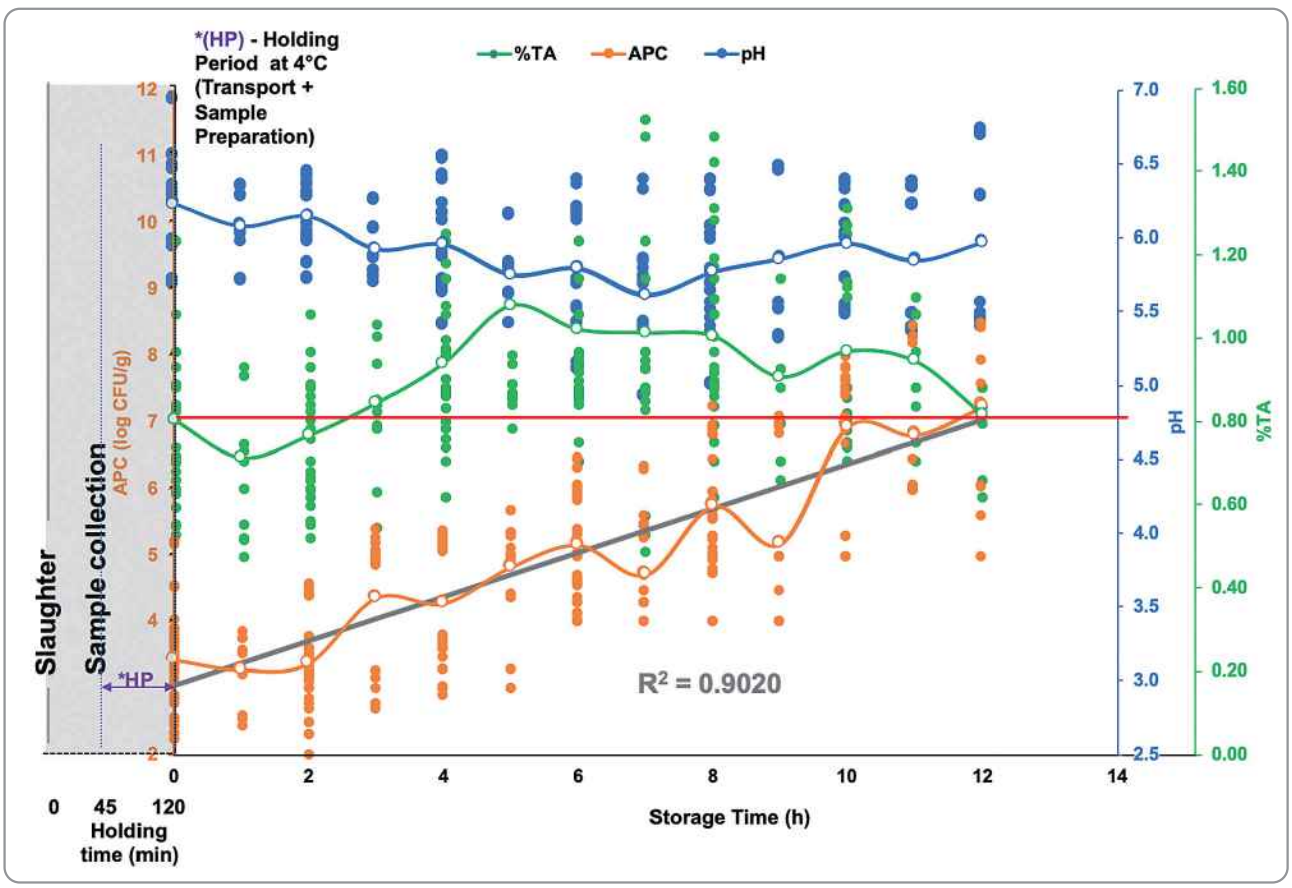


**Figure 1.** Aerobic plate count (APC), pH and titratable acidity (%TA) of pork meat stored at ambient temperature from 0 to 12 h obtained from eleven (11) sampling runs with three internal replicates per each run. Average values of each parameter are represented by unfilled markers within the curves with similar colour obtained per storage time. The grey curve represents the APC population fit in the *Baranyi and Roberts* (1994) model.

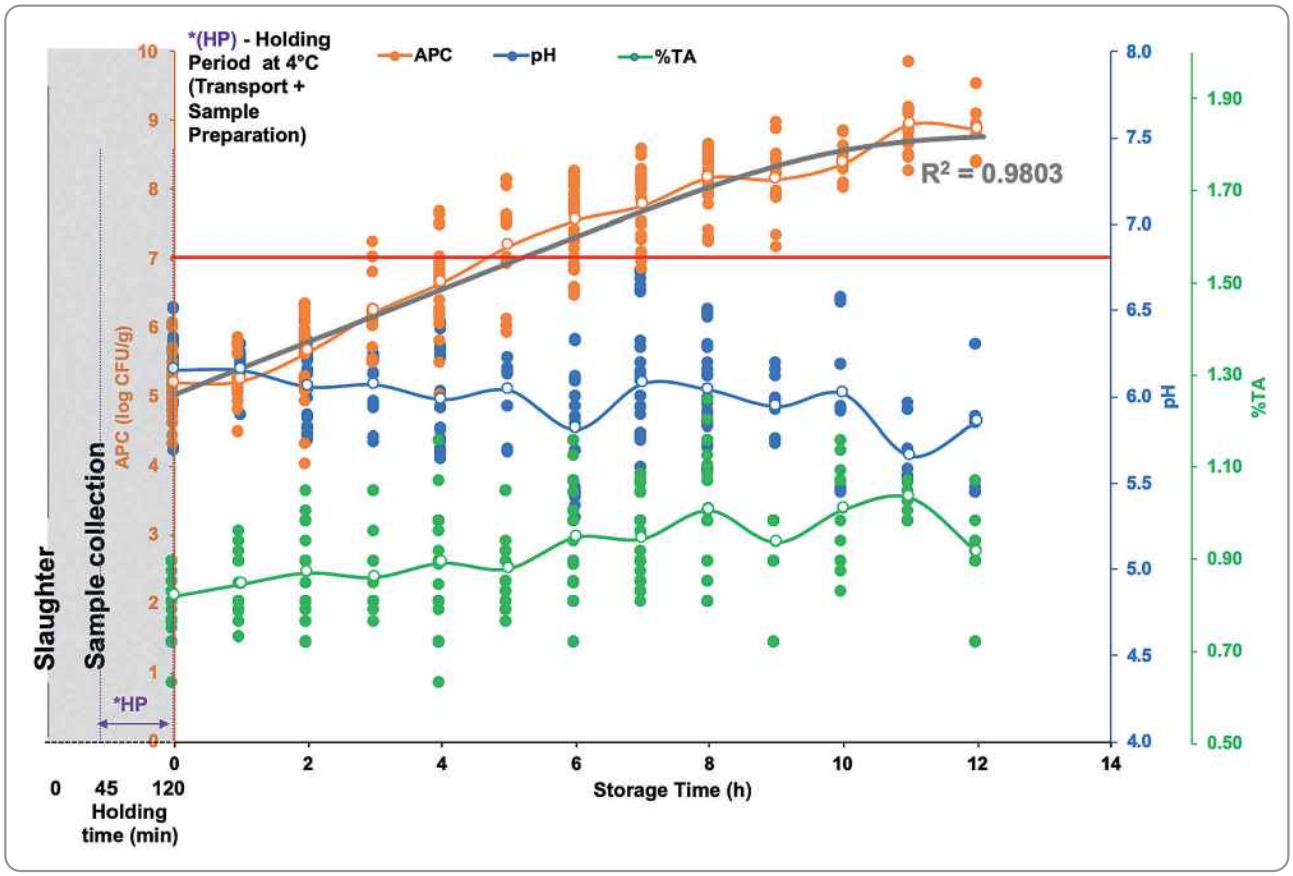


**Figure 2.** Aerobic plate count (APC), pH, and titratable acidity (%TA) of chicken meat stored at ambient temperature from 0 to 12 h obtained from ten (10) sampling runs with three internal replicates per each run. Average values of each parameter are represented by unfilled markers within the curves with similar colour obtained per storage time. The grey curve represents the APC population fit in the *Baranyi and Roberts* (1994) model.

of both meats were comparable to the acceptable meat quality of newly slaughtered pork (*Boler et al.*, 2010) and chicken (*Ristic & Dame*, 2010) meats. On the other hand, %TA is not commonly measured in newly slaughtered meat, but based on the studies of *van Laack* (2000) and *Terefe* (2017), fresh meat with pH of 5.56±0.12 and 5.71±0.05 had %TA of 1.4±0.2% and 0.96±0.17%, respectively.

pH and % TA of both meats in the present study were variable during storage. Generally, a decreasing trend was observed for pH but an increasing trend for %TA; however, the changes in both parameters were not significant throughout the storage period of pork (Table 1) and chicken (Table 2) meats. These results did not show similar trends to several studies conducted previously. In the study of *Choi et al.* (2017), the pH of pork meat stored aerobically at room temperature for 48 h and monitored every 4 h increased during storage. In other studies that used different storage conditions, the trends in pH increased whereas the trends in titratable acidity decreased during storage, regardless of whether the meats were pork or chicken (*Golasz et al.,* 2013; *Kuswandi et al.,* 2014; *Singh, Sahoo, Chatli & Biswas*, 2014; *Terefe*, 2017). The difference of the present results from the previous studies could be due to differences in setting the time for the initial readings of pH and %TA in meat and the monitoring duration and intervals.

The generally decreasing trends in pH and increasing trends in %TA of pork and chicken meats in the present study could be attributed to the conversion of available glucose into organic acids by lactic acid bacteria (LAB) (*Hernandez-Herrero et al*., 1999; *Fraqueza et al.*, 2008), a group of spoilage bacteria that is commonly present in meat carcasses (*Chouliara et al.*, 2008; *Patsias et al.*, 2008). There is a possibility that the pH of pork and chicken meats in the present study would also increase if stored for longer durations, as was observed by *Choi et al*. (2017) in their study. This commonly happens when the growth of *Pseudomonas* overtakes that of LAB, causing increased production of ammonia and other products of amino acid decomposition

**Table1.** Titratable acidity, pH, and aerobic plate count (APC) of pork meat stored under ambient temperature for 12 hours

| Storage time (hours) | Titratable acidity (% lactic acid) | | | pH | | | APC (log CFU/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean±SD | Min | Max | Mean±SD | Min | Max | Mean±SD | Min | Max |
| Initial | 0.81±0.19$^{bcd}$ | 0.55 | 1.28 | 6.22±0.37$^{a}$ | 5.67 | 6.93 | 3.42±0.76$^{ef}$ | 2.23 | 5.23 |
| 1 | 0.70±0.17$^{d}$ | 0.49 | 0.97 | 6.07±0.26$^{ab}$ | 5.69 | 6.34 | 3.27±0.50$^{g}$ | 2.43 | 3.85 |
| 2 | 0.77±0.15$^{cd}$ | 0.54 | 1.10 | 6.14±0.25$^{ab}$ | 5.70 | 6.53 | 3.37±0.56$^{g}$ | 2.00 | 4.57 |
| 3 | 0.85±0.14$^{bcd}$ | 0.56 | 1.07 | 5.92±0.20$^{abc}$ | 5.68 | 6.25 | 4.35±1.04$^{cde}$ | 2.70 | 5.41 |
| 4 | 0.94±0.17$^{abcd}$ | 0.64 | 1.30 | 5.95±0.34$^{abc}$ | 5.39 | 6.54 | 4.27±0.96$^{def}$ | 2.90 | 5.39 |
| 5 | 1.08±0.35$^{a}$ | 0.81 | 1.79 | 5.75±0.25$^{bc}$ | 5.40 | 6.14 | 4.82±0.84$^{bcd}$ | 3.00 | 5.69 |
| 6 | 1.03±0.26$^{ab}$ | 0.73 | 1.79 | 5.79±0.37$^{bc}$ | 5.09 | 6.38 | 5.15±0.84$^{bc}$ | 4.00 | 6.50 |
| 7 | 1.02±0.30$^{ab}$ | 0.50 | 1.59 | 5.61±0.42$^{c}$ | 4.91 | 6.37 | 4.70±0.58$^{bcd}$ | 4.00 | 5.60 |
| 8 | 1.01±0.21$^{ab}$ | 0.64 | 1.54 | 5.77±0.39$^{bc}$ | 4.99 | 6.37 | 5.74±0.98$^{b}$ | 4.00 | 7.26 |
| 9 | 0.91±0.15$^{abcd}$ | 0.68 | 1.19 | 5.86±0.51$^{abc}$ | 5.30 | 6.47 | 5.17±1.20$^{b}$ | 4.00 | 7.11 |
| 10 | 0.97±0.22$^{abc}$ | 0.73 | 1.36 | 5.96±0.34$^{abc}$ | 5.46 | 6.38 | 6.93±0.95$^{a}$ | 5.00 | 8.01 |
| 11 | 0.95±0.15$^{abc}$ | 0.73 | 1.14 | 5.84±0.45$^{abc}$ | 5.35 | 6.36 | 6.80±1.08$^{a}$ | 6.00 | 8.49 |
| 12 | 0.82±0.11$^{bcd}$ | 0.64 | 0.91 | 5.97±0.56$^{abc}$ | 5.38 | 6.72 | 7.23±1.39$^{a}$ | 5.00 | 8.53 |

**Note:** Different letters within a column indicate significant differences ($p<0.05$)

**Table 2.** Titratable acidity, pH, and aerobic plate count (APC) of chicken meat stored under ambient temperature for 12 hours

| Storage time (hour) | Titratable acidity (% lactic acid) | | | pH | | | APC (log CFU/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean±SD | Min | Max | Mean±SD | Min | Max | Mean±SD | Min | Max |
| Initial | 0.82±0.07$^{d}$ | 0.63 | 0.90 | 6.15±0.22$^{a}$ | 5.67 | 6.49 | 5.20±0.49$^{g}$ | 4.30 | 6.00 |
| 1 | 0.85±0.07$^{cd}$ | 0.73 | 0.97 | 6.15±0.14$^{a}$ | 5.87 | 6.28 | 5.23±0.36$^{g}$ | 4.48 | 5.84 |
| 2 | 0.87±0.09$^{cd}$ | 0.72 | 1.06 | 6.05±0.18$^{ab}$ | 5.73 | 6.30 | 5.65±0.53$^{g}$ | 4.00 | 6.33 |
| 3 | 0.86±0.09$^{cd}$ | 0.76 | 1.06 | 6.07±0.18$^{ab}$ | 5.72 | 6.22 | 6.23±0.52$^{f}$ | 5.48 | 7.23 |
| 4 | 0.89±0.11$^{cd}$ | 0.63 | 1.17 | 5.98±0.26$^{ab}$ | 5.62 | 6.40 | 6.65±0.55$^{f}$ | 5.00 | 7.67 |
| 5 | 0.88±0.10$^{cd}$ | 0.77 | 1.06 | 6.03±0.19$^{ab}$ | 5.66 | 6.20 | 7.18±0.68$^{e}$ | 5.90 | 8.13 |
| 6 | 0.95±0.12$^{abc}$ | 0.72 | 1.17 | 5.81±0.31$^{bc}$ | 5.28 | 6.31 | 7.55±0.56$^{de}$ | 6.45 | 8.26 |
| 7 | 0.95±0.09$^{abc}$ | 0.81 | 1.10 | 6.07±0.35$^{ab}$ | 5.54 | 6.70 | 7.76±0.50$^{cd}$ | 6.85 | 8.57 |
| 8 | 1.01±0.14$^{ab}$ | 0.81 | 1.26 | 6.04±0.30$^{ab}$ | 5.55 | 6.48 | 8.16±0.35$^{bc}$ | 7.23 | 8.63 |
| 9 | 0.94±0.08$^{abc}$ | 0.72 | 0.99 | 5.94±0.15$^{abc}$ | 5.71 | 6.17 | 8.14±0.50$^{bc}$ | 7.14 | 8.95 |
| 10 | 1.01±0.12$^{ab}$ | 0.83 | 1.17 | 6.01±0.42$^{ab}$ | 5.43 | 6.55 | 8.37±0.25$^{b}$ | 8.01 | 8.82 |
| 11 | 1.04±0.04$^{a}$ | 0.99 | 1.08 | 5.66±0.17$^{c}$ | 5.49 | 5.94 | 8.93±0.44$^{a}$ | 8.25 | 9.83 |
| 12 | 0.92±0.08$^{bcd}$ | 0.72 | 1.08 | 5.85±0.31$^{bc}$ | 5.43 | 6.28 | 8.87±0.40$^{a}$ | 8.36 | 9.51 |

(*Hernandez-Herrero et al.,* 1999; *Masniyom et al.*, 2002; *Fraqueza et al.,* 2008). This notion can be supported by the locally conducted study of *Pel et al.* (2017), where fresh pork from a wet market was monitored for 30 h every 5 h. The initial pH of pork meat in their study was 6.13 which decreased to 6.06 after 10 h of storage. Reversal in the pH trend began at 15 h, and it continuously increased until 30 h. Similar to the present study, although the pH of pork initially decreased after 10 h of storage in the study of *Pel et al.* (2017), the decrease was not significant.

Aside from microbiological activity, the minimal and insignificant changes of pH and %TA in meat in the present study can also be explained by several biochemical reactions involved in the conversion of muscle to meat. The normal muscle pH of a live animal is close to neutral, and ranges from 6.5 to 6.8 (*Pel et al*., 2017), but can reach up to 7.2 to 7.3 in some cases (*Knox*, 2003). Immediately after slaughter, anaerobic glycolysis begins and the stored glycogen in the muscle is converted into lactic acid, leading the muscle pH to decrease (*Bruckner*, 2010). Muscle pH falls steadily from slaughter until the muscle runs out of energy or the pH is too low for enzymatic activity and the rigor is complete (*Marsh*, 1981). At the post-rigor stage, all reserve glycogen in the muscle has already been used up, and therefore, lactic acid formation would be ceased, maintaining the pH level and %TA of meat for a certain period of time. In addition, *Nielsen and Nielsen* (2012) discussed that the meat pH, after glycolysis has been completed, depends not only on the lactate concentration but also on the pH buffer capacity of the tissue. In pork and chicken meats, the buffer capacity of the muscle tissue originates primarily from carnosine (β-alanyl-l-histidine). Carnosine is a dipeptide that can neutralize the lactic acid formed when the degradation of glycogen exceeds the capacity of the Krebs cycle (*Abe*, 2000). These could be the possible reasons why the pH and %TA of the post rigor meat samples in the present study did not change significantly throughout storage.

Another important quality indicator in meat is APC. It represents the largest group of microorganisms enumerated in food and serves as an indicator of the overall level of contamination in fresh pork (*Knox*, 2003). The initial APC levels of our pork meat ranged from 2.23 to 5.23 log CFU $g^{-1}$, while the initial APC levels of chicken were 4.30 to 6.0 log CFU $g^{-1}$ (Tables 1 and 2). The APC levels were in agreement with the ranges reported by other works for animal carcasses slaughtered under appropriate hygienic conditions (*West et al.*, 1972; *Göksoy et al.*, 2004; *Zhang et al.,* 2012). The microbial population increased over storage time, reaching 5.0 to 8.53 log CFU $g^{-1}$ for pork (Figure 1) and 8.36 to 9.51 log CFU $g^{-1}$ for chicken (Figure 2) at the end of 12 h storage. Pork and chicken meat samples reached the end of shelf-life between 8 to 12 h and between 3 to 6 h of storage, respectively, after attaining the average APC value of 7.0 log CFU $g^{-1}$, which was previously considered as the upper acceptability limit for fresh meat *(Senter et al.*, 2000). The APC of pork obtained in this study agrees with that reported in the study of *Tejada et al*. (2013). Based on their study, newly slaughtered meat with initial APC of 3.81 reached 7.0 log CFU $g^{-1}$ at 10 h. On the other hand, chicken meat reached the average APC value of 7.0 log CFU $g^{-1}$ after 5 h of storage but there were a few samples that already reached the end of shelf-life after as little as 3 h of storage.

Local regulations allow the holding of newly slaughtered meat in the wet market for 8 h (*NMIS*, 2012). In the current study, the microbial shelf-life of the newly slaughtered pork was still acceptable after 8 h storage, but that of chicken was unacceptable. Although the present study in our laboratory setting found newly slaughtered pork meat can maintain an acceptable microbial level until 8 to 12 h post slaughter, another local study reported the microbial count of meat obtained from a wet market was already unacceptable after 5 h of storage at ambient temperature (*Pel et al.*, 2017).

### *Relationship of pH, APC and %TA in pork and chicken meats*

Relationships between pH, APC and %TA in pork and chicken meats were determined using Pearson correlation coefficient as shown in Tables 3 and 4, respectively. %TA had a direct opposite trend to that of meat pH, as was observed in some other studies (*Singh et al.,* 2014; *Terefe*, 2017). Several studies show that pH can be used for monitoring of meat shelf-life due to its relationship with microbial spoilage (*Mano et al.*, 2002; *Muela et al.*, 2010; *Kanatt et al.*, 2010; *Golasz et al.*, 2013). There was a significant negative relationship between pH and %TA of pork ($r = -0.593$, $n = 288$, $p < 0.005$) and between pH and %TA of chicken ($r = -0.338$, $n = 281$, $p < 0.005$). Although these results somewhat support the previous studies of *Singh et al.* (2014) and *Terefe* (2017), they also negate the result of *van Laack* (2000). According to the Department of Food, Bioprocessing and Nutrition Sciences (FBNS, n.d), there is no fixed relationship between pH and titratable acidity

**Table 3.** Pearson correlation of %TA, pH and aerobic place count (APC) of pork meat

| | | %TA | pH | APC (log CFU/g) |
|---|---|---|---|---|
| **%TA** | Pearson Correlation | 1 | −.593** | .053 |
| | Sig. (2-tailed) | | .000 | .376 |
| | N | 288 | 288 | 278 |
| **pH** | Pearson Correlation | −.593** | 1 | –.010 |
| | Sig. (2-tailed) | .000 | | .865 |
| | N | 288 | 288 | 278 |
| **APC (log CFU/g)** | Pearson Correlation | .053 | −.010 | 1 |
| | Sig. (2-tailed) | .376 | .865 | |
| | N | 278 | 278 | 342 |

**Legend:** **Correlation is significant at the 0.01 level (2-tailed).

**Table 4.** Pearson correlation of pH, titratable acidity (%TA) and aerobic plate count (APC) of chicken meat

| | | pH | %TA | APC (log CFU/g) |
|---|---|---|---|---|
| **pH** | Pearson Correlation | 1 | −.338** | −.165** |
| | Sig. (2-tailed) | | .000 | .007 |
| | N | 282 | 281 | 267 |
| **%TA** | Pearson Correlation | −.338** | 1 | .419** |
| | Sig. (2-tailed) | .000 | | .000 |
| | N | 281 | 281 | 266 |
| **APC (log CFU/g)** | Pearson Correlation | −.165** | .419** | 1 |
| | Sig. (2-tailed) | .007 | .000 | |
| | N | 267 | 266 | 342 |

**Legend:** **Correlation is significant at the 0.01 level (2-tailed).

in a food; instead, the pH is influenced by the ability of the acids present to dissociate (*Neta et al.*, 2007).

No statistically significant relationships were found between APC and pH ($r$=−0.10, $n$=278, $p>0.05$) and between APC and %TA ($r$=0.053, $n$=278, $p>0.05$) of pork. These findings agree with *Bruckner* (2010) and *Pel et al.* (2017). Contrariwise, there was a weak negative relationship between APC and pH ($r$=−0.165, $n$=267, $p<0.005$) in chicken and a positive relationship between APC and %TA (r=0.419, n=266, $p<0.005$). This indicates that pH cannot be used as a good indicator of meat spoilage. Moreover, the differences between fresh and obviously spoiled meat in the present study, for both pH and %TA, were not great enough for practical use. These impressions were demonstrated by the statistically treated average values of pH, APC, and %TA, as shown in Table 1 for pork and Table 2 for chicken. Results of ANOVA showed that the pH and %TA of pork meat stored for 8 to12 h (spoiled meat) were not significantly different ($p>0.05$) to the same parameters of some of our pork meat stored for 0 to 7 h (acceptable meat). Similarly, pH and %TA of chicken meat stored for 3 to 12 h (spoiled meat) showed no significant difference ($p>0.05$) to these parameters in some of the chicken meat stored for 0 to 2 h (acceptable meat). However, APC levels in spoiled pork and chicken meats were significantly different ($p<0.05$) from those in meats that were still acceptable for consumption.

## Conclusion

As a general evaluation, the microbial populations of pork and chicken meats both increase during storage for 12 hours at ambient temperature, while pH and %TA of the meats are not significantly affected by this storage. Relationships between APC and physicochemical characteristics of both meats are weak. Therefore, developing microbial spoilage indicators based on either pH or %TA for meat may not be feasible, and based on this study, the only way to determine the shelf-life of meat is to conduct microbiological analysis. In terms of microbiological shelf-life (APC <7 log CFU $g^{-1}$), a suitable pork shelf-life is attained when the local regulation of maximum holding time of 8 h at ambient temperature is conformed with, while some chicken meat can reach the end of its shelf-life in as little as 3 h storage at ambient temperature, showing non-conformity. Research related to shelf-life determination of newly slaughtered meat, particularly chicken, at ambient temperature is very scanty and rare. This may be due to the fact that holding fresh meat at ambient temperature is not widely accepted in other countries. With that, conduct of related study is encouraged to gather more information on meat spoilage occurrence in local scenarios. Moreover, development of affordable methods as alternatives to chilling to extend the shelf-life and overall safety of raw meat are necessary, especially for developing countries that practice the same method of meat handling as described in this study.

# Promene u fizičko-hemijskim i mikrobiološkim svojstvima svinjskog i pilećeg mesa u uslovima ambijentalnog skladištenja

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*A p s t r a k t : Uzorci svinjskog i pilećeg mesa su uzeti iz prethodno odabranih klanica kako bi se odredila pH vrednost, % TA i broj aerobnih bakterija (Aerobic Plate Count — APC) od vremena klanja do isteka roka trajanja na sobnoj temperaturi (30±2°C). Rezultati su pokazali da se populacija mikroorganizama u uzorcima mesa povećavala tokom vremena skladištenja. Sa druge strane, pH vrednost i% TA su varirali i nisu pokazali statistički značajne promene tokom perioda skladištenja. Na osnovu mikrobiološke analize, rok trajanja svinjskog i pilećeg mesa kretao se u rasponu od 8 do 12 h, odnosno 3 do 6 h. Pearsonova korelacija otkrila je da ne postoji značajna veza između broja aerobnih bakterija i pH vrednosti svinjetine ($r = -0,10$; $n = 278$, $p > 0,05$) i između broja aerobnih bakterija i % TA svinjetine ($r = 0,053$; $n = 78$, $p > 0,05$). S druge strane, postojala je slaba negativna korelacija između broja aerobnih bakterija i pH vrednosti kod piletine ($r = -0,165$; $n = 267$, $p < 0,005$) i pozitivna između broja aerobnih bakterija i % TA ($r = 0,401$; $n = 66$, $p < 0,005$). To je pokazalo da se pH vrednost ne može koristiti kao dobar pokazatelj kvarenja mesa. Pored toga, razlike između uzoraka svežeg i očigledno pokvarenog mesa, za pH vrednost, kao i za % TA, nisu bile dovoljno velike za praktičnu upotrebu.*

***Ključne reči:*** *svinjetina, piletina, pH, broj aerobnih bakterija, titrabilna kiselost.*

**Disclosure statement:** No potential conflict of interest was reported by authors.

**Acknowledgments:** This study is partially supported by the Science Education Institute of the Department of Science and Technology (DOST-SEI) through its DOST-Human Resource Development Program. The authors would also like to thank Dr. Eriq de Gula, the Head of Meat Inspection Service, Valenzuela City for the assistance in the collection of meat samples.

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Paper received: May 11th 2020.
Paper corrected: June 18th 2020.
Paper accepted: June 16th 2020.

**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 641.1:637.5*
*ID: 16234249*
*https://doi.org/10.18485/meattech.2020.61.1.4*

*Original scientific paper*

# Nutritional score of meat products at retail in Serbia

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*A b s t r a c t: Nutri-score is simplified front-of-pack nutrition labelling on packed food products, used in a broad international context to categorize food products into five colour/letter grades (best A to worst E) that reflect the foods' nutritional quality. The labels serve as potential help for consumers to make healthier food choices and encourage food industries to improve the nutritional quality of the foods they produce. The aim of this study was to determine the nutritional scores and grades of meat products present on the Serbian retail market. Ultimately, this should point out to consumers the importance of proper nutrition and encourage the meat industry to adapt to new labelling requirements. During a two year period, 310 packaged locally-produced meat products were purchased at retail, graded according to the Nutri-score method and categorized into 13 ad hoc product groups. The results obtained showed that 82.5% of all examined meat products had nutritional scores that meant they were classified as unhealthy foods, while only 2.9% of meat products (these were fresh meat or minced meats) were classified as healthy foods. Of the total number of examined meat products, 41.5% were classified as grade E, 41% were classified as grade D, 13% received grade C, 1.6% received grade B, while only 2.9 % were classified as grade A. Sodium chloride was an especially burdensome parameter in 10 groups (77% of all products examined), while the presence of saturated fat was troublesome in 7 groups (54%) and high energy balance in 2 groups (15%). Serbian manufacturers are advised to implement new formulations and/or procedures in an effort to reduce these parameters in the meat products they produce.*

***Keywords:*** *nutritional score, meat products, nutrition labelling, sodium chloride, healthy food.*

## Introduction

Novel national legislation (*Anonymous*, 2018) requires a nutrition declaration on meat products placed on the Serbian retail market. In the European Union, labelling of nutritional parameters has been mandatory since 2011 (*Anonymous*, 2011). In 2008, following an evaluation of the legislation on food labelling by the European Commission's Directorate-General for Consumer and Health, the European Commission issued a proposal which would combine two major Directives (Directive 2000/13/EC1 and Directive 90/496/EEC2) into one Framework Regulation (Regulation 1169/2011) (*Anonymous*, 1990, 2000, 2011). Areas covered by the Regulation are, amongst others, nutrition information, origin labelling, legibility and allergen labelling. For prepacked foods, food business operators must have a nutrition declaration on their label, indicating the energy value and the amounts of fat, saturated fat, carbohydrate, sugar, protein and salt. The energy value and all nutrients declared must be expressed in absolute amounts per 100 g or 100 ml (*Anonymous*, 2013). They can also be expressed per package or per portion. Information on vitamins and minerals must, in addition, be expressed as a percentage of the nutrient reference values (NRVs), which can also be given in graphic form (*Anonymous*, 2011c). From December 2016, food in the European Union had to be labelled with this nutritional data. Since the actual nutritional value of food can vary in relation to the declared value, it is important to define and specify the average nutritional value of the product (*Knezevic and Rimac-Brncic*, 2014). Consumer groups and public health organisations have called for bans on the advertising of "unhealthy" food to children for several decades. However, the definition of unhealthy has been a topic of considerable argument. Food companies have resisted having any products described as unhealthy, but have gradually developed a number of different schemes that define products they believe are 'healthy' (or at least 'healthier') and appropriate for advertising to children. Health and consumer groups have called for a single scheme — or nutrient profiling model — consistent with international recommendations for

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**Figure 1.** Nutri-score nutrition labelling scheme on the front food label at retail

preventing chronic disease and with national food-based dietary guidelines. Ideally, this is a simple system which could be applied to all products and with a clearly defined cut-off for defining which foods are not suitable for advertising to children (*Rayner and Scarborough*, 2009).

Front-of-pack nutrition labels are designed to help consumers evaluate the healthiness of foods and to promote healthier food choices (*Hagmann and Siegrist*, 2020). Since the beginning of the 20$^{th}$ century, the rise of unhealthy dietary habits has been a trend in many countries (WHO, 2003). Much of the disease burden worldwide, including cancers, cardiovascular disease and diabetes, could be reduced if people changed their behaviours, e.g. stopped smoking, reduced their alcohol intake, ate healthier diets and became more physically active (*Diepeveen et al*., 2013). Policy makers have a variety of means at their disposal by which they can try to influence these behaviours, ranging from the provision of information to the public, through to measures that restrict choice by regulation (*Anonymous*, 2007).

The Nutri-score label is based on the United Kingdom (UK) Food Standards Agency's nutrient profiling system (NPS; original version: *FSA-NPS*, 2011), which evaluates the overall healthiness of a food product according to its nutritional composition (*Anonymous*, 2011b). The FSA-NPS was built with a perspective of preventing a large range of chronic diseases. It allocates a score to a given food/beverage from its content (per 100 g or 100 ml) of energy, saturated fat, sugar, sodium, dietary fibre, protein and fruit/vegetables/legumes/nuts. The model applies equally to all food and drink, there are no exemptions or category-specific criteria, and a judgment can be made as to whether the food should be placed under advertising restrictions (Anonymous, 2011a). It was initially developed and validated in the UK, where it has been used for advertising regulation (*Arambepola et al.*, 2008) and was transposed in France as FSAm-NPS (*Julia et al*., 2014). To classify healthiness, the product's content of several health-promoting (fruit, vegetables and nuts, fibre and protein) and critical nutrients (energy, saturated fat, total sugar and sodium) is evaluated. This results in a single NPS, which is then transformed into a colour-coded letter-based grade that is simple for consumers to understand (Figure 1). Studies of the NPS underlying Nutri-score, and comparative studies of the perception, understanding and use of various strategies for front-of-pack labelling, done between 2014 and 2017, concluded that the Nutri-score was superior to other formats (*Julia and Hercberg*, 2017). The scientific evidence weighted heavily in the decision by health authorities to adopt the Nutri-score in France (*Julia et al*., 2018).

Consumption of more food with higher FSAm-NPS Dietary Index (DI) scores, reflecting a lower nutritional quality of the food consumed, was associated with an increased risk of cancer (colo-rectal, upper aerodigestive tract and stomach, liver, and lung in men and breast in women) (*Deschasaux et al*, 2019). A nutritional scoring system is helpful to consumers, because it encourages them to decide to purchase more adequately healthy foods (in Nutri-score, foods with these scores are graded as A or B). Consumption of food products with worse nutritional grades (D or E) was associated with a higher risk of development of chronic diseases and cancer in a large multinational European cohort. Since 31 October 2017, the Nutri-score front-of-pack labelling system (a synthetic information system based on colours and letters from green/A to red/E), allowing consumers to see and compare at a glance the nutritional value of pre-packaged foods, has been implemented on a voluntary basis in France (*Anonymous*, 2017).

## Materials and Methods

A total of 310 packaged meat products, locally produced in Serbia and sold on the Serbian retail market, were examined over a period of two years. Meat products were in their original packaging,

taken from retail, and were classified by us into 13 *ad hoc* groups:

1. Boiled sausages finely ground
2. Boiled sausages coarsely ground
3. Boiled sausages with meat pieces
4. Cooked sausages and pâtés
5. Canned minced meat
6. Canned meat chunks
7. Smoked meat products
8. Dried meat products
9. Dried and semi-dried fermented sausages
10. Meat dishes and dishes with meat
11. Bacon and crackling
12. Meat preparations
13. Fresh meat and minced meat.

The sampled meat products, meat preparations and fresh meat were originally from domestic producers. On the basis of the information given in the nutrition declaration, the meat products were scored according to Nutrient Profiling Technical Guidance (*Anonymous*, 2011a).

In brief, the model uses a simple scoring system where points are allocated on the basis of the nutrient content of 100 g of a food or drink. Points were awarded for category A nutrients (energy, saturated fat, total sugar and sodium; Table 1) and for category C nutrients (fruit, vegetable and nut content, fibre and protein; Table 2). The score for category C nutrients was the subtracted from the score for category A nutrients to give the final nutrient score. Fibre content was calculated as shown in generic Table 2, using the AOAC reference method.

For category A nutrients, a maximum of ten points can be awarded for each nutrient. For category C nutrients, a maximum of five points can be awarded for each nutrient/food component. After obtaining the final nutrition scores, the values were compared with the cut-off values for the colour-coded food grades A to E (Table 3).

**Table 1.** Class A nutrient point scores, depending on the amount of each nutrient in 100 g or 100 ml of a food or drink

| Points | Energy (kJ) | Saturated Fat (g) | Total Sugar (g) | Sodium (mg) |
|---|---|---|---|---|
| 0 | ≤ 335 | ≤ 1 | ≤ 4.5 | ≤ 90 |
| 1 | > 335 | > 1 | > 4.5 | > 90 |
| 2 | > 670 | > 2 | > 9 | > 180 |
| 3 | > 1005 | > 3 | > 13.5 | > 270 |
| 4 | > 1340 | > 4 | > 18 | > 360 |
| 5 | > 1675 | > 5 | > 22.5 | > 450 |
| 6 | > 2010 | > 6 | > 27 | > 540 |
| 7 | > 2345 | > 7 | > 31 | > 630 |
| 8 | > 2680 | > 8 | > 36 | > 720 |
| 9 | > 3015 | > 9 | > 40 | > 810 |
| 10 | > 3350 | > 10 | > 45 | > 900 |

**Table 2**. Class C nutrient point scores, depending on the amount of each nutrient in 100 g or 100 ml of a food or drink

| Points | Fruit, vegetables, nuts (%) | Fibre (g), measured as non-starch polysaccharides | Fibre (g), measured by the AOAC[a] method | Protein (g) |
|---|---|---|---|---|
| 0 | ≤ 40 | ≤ 0.7 | ≤ 0.9 | ≤ 1.6 |
| 1 | > 40 | > 0.7 | > 0.9 | > 1.6 |
| 2 | > 60 | > 1.4 | > 1.9 | > 3.2 |
| 3 | – | > 2.1 | > 2.8 | > 4.8 |
| 4 | – | > 2.8 | > 3.7 | > 6.4 |
| 5 | > 80 | > 3.5 | > 4.7 | > 8.0 |

[a] American Association of Oil Chemists

**Table 3.** Limit values of the nutritional score for determining the color-based grade to which a food belongs

| Total nutritional score for solid foods | Total nutritional score for liquid foods | Colour and matching letter |
|---|---|---|
| To –1 | 0 | Dark green – A |
| 0–2 | Minimum 1 | Light green – B |
| 3–10 | 2–5 | Yellow – C |
| 11–18 | 6–9 | Light orange – D |
| 19 and more | 10 and more | Dark orange – E |

**Source:** Nutri-score — The front of pack nutrition labelling scheme recommended in France (https://ec.europa.eu/food/sites/food/files/animals/docs/comm_ahac_20180423_pres4.pdf)

If a food or drink scores 11 or more category A points, then it cannot score points for protein unless it also scores 5 points for fruit, vegetables and nuts. A food is classified as less healthy if it scores 4 points or more. Therefore, in terms of the presence of nutrients in 100 g or 100 ml, foods that are labelled green (A or B) are considered to be of better health quality, while foods that are labelled orange (D or E) are of lower health quality.

## Results

Grouped results of the colour-coded food grades achieved by locally-produced meat products on the Serbian retail market are presented in Table 4.

The results showed that 82.5% of all examined meat products were classified as unhealthy foods, while only 2.9% of the meat products (and these were fresh meats and minced meats) were classified as healthy foods.

**Table 4.** Number of meat products in each nutritional grade for groups of meat products available at retail level in Serbia

| Meat product group | Number of samples examined | Nutritional grade | | | | |
|---|---|---|---|---|---|---|
| | | A | B | C | D | E |
| Boiled sausages, finely ground | 30 | | | | 20 | 10 |
| Boiled sausages, coarsely ground | 30 | | | | 8 | 22 |
| Boiled sausages with meat pieces | 20 | | | 5 | 15 | |
| Canned minced meat | 20 | | | 5 | 12 | 3 |
| Canned meat chunks | 30 | | | 11 | 19 | |
| Smoked meat products | 30 | | 2 | 7 | 19 | 2 |
| Dried meat products | 20 | | | | 10 | 10 |
| Dried and semi-dried fermented sausages | 30 | | | | | 30 |
| Pâté and cooked sausages | 20 | | | 1 | 12 | 7 |
| Meat dishes and dishes with meat | 20 | 1 | 1 | 9 | 8 | 1 |
| Bacon and cracklings | 30 | | | | | 30 |
| Meat preparations | 20 | | | 2 | 4 | 14 |
| Fresh meat and minced meats | 10 | 8 | 2 | | | |
| **Total** | **310** | **9** | **5** | **40** | **127** | **129** |
| **%** | **100** | **2.9** | **1.6** | **13** | **41** | **41.5** |

**Table 5.** Particularly burdensome nutritional parameters found in meat products available at retail in Serbia

| Meat product group | Particularly burdensome nutritional parameters |
|---|---|
| Boiled sausages finely ground | Sodium chloride, saturated fat |
| Boiled sausages coarsely ground | Sodium chloride, saturated fat |
| Boiled sausages with meat pieces | Sodium chloride |
| Canned minced meat | Sodium chloride, saturated fat |
| Canned meat chunks | Sodium chloride |
| Smoked meat products | Sodium chloride |
| Dried meat products | Sodium chloride |
| Dried and semi-dried fermented sausages | Saturated fat, sodium chloride, energy |
| Pâté and cooked sausages | Saturated fat |
| Meat dishes and dishes with meat | Sodium chloride |
| Bacon and cracklings | Saturated fat, energy |
| Meat preparations | Saturated fat, sodium chloride |
| Fresh meat and minced meats | None detected |

Particularly burdensome nutritional parameters that led to the classification of meat products as unhealthy food are presented in Table 5.

Particularly burdensome parameters significantly contributed to the formation of the final assessment of nutritional scores (data not shown) for meat products. For the 13 groups of examined meat products for which the nutritional score was determined, sodium/sodium chloride was an especially burdensome parameter in 10 groups (77%), while the presence of saturated fat was troublesome in 7 groups (54%) and a high energy balance in 2 groups (15%).

## Discussion

Analysis of the nutritional score for meat products on the Serbian retail market shows that 82.5% of all examined meat products were classified as unhealthy foods, while only 2.9% of the meat products (and these were fresh meats or minced meats) were classified as healthy foods. Especially burdensome parameters for the nutritional scores were sodium chloride (77%), saturated fat (54%) and high energy balances (15%). Reasons for this classification of meat products as mostly unhealthy can lie in the nature of the foods themselves, if these are not intended to be consumed in quantities as large as 100 g per day (bacon, dried meat products, fermented sausages, pâté). However, for other meat products examined, recipe corrections will be needed in order to reduce burdensome nutrients. First of all, the need to reduce sodium in meat products is evident, as many groups of meat products were classified as unhealthy foods precisely because of their high sodium content (boiled sausages, canned minced meat and meat chunks, smoked meat products, dried meat products, fermented sausages, meat dishes and meat preparations). A previous market study in Serbia also reported the need to reduce sodium in many groups of meat products (*Lilic et al*., 2017).

Among the additives used in the production of meat products, kitchen salt (sodium chloride) is the most commonly used, giving a desirable taste, improved texture and prolonged shelf-life. Salt is added to meat products primarily to produce the characteristic, necessary salty taste of meat products. The degree of sensory perception of salt depends not only on the percentage/ratio of salt to other product components, but also on the degree of hydration and quantity of water (*Raseta et al.*, 2013). Although sodium chloride is a very important food ingredient, it is commonly found in large quantities (%) in meat products (1.2–1.8% in cooked sausages, 1.8–2.2% in both finely and coarsely ground boiled sausages, 1.2–2.5% in canned minced meat, 2.4–3.0% in fermented sausages and 3.0–5.0% in dried meat products) (*Vukovic*, 2012). Daily consumption of meat products with high levels of sodium invokes increased blood pressure and consequent cardiovascular disorders in consumers. Therefore, there is an intense effort to reduce consumers' daily sodium intake (*Lazic et al.*, 2015).

Saturated fat content was also a burdensome nutritional score parameter for 54% of our examined meat products, together with a high energy balance (15%). Daily energy intake from saturated fat should be in the range of 5–10% of all-body energy needs, while trans fatty acids (industrial and those occurring naturally in food) should contribute <1% of total daily energy needs (*Spajic*, 2020). Eating habits and nutritional values of foods play key roles in the prevention of major chronic degenerative diseases (*Kant*, 2010). Helping consumers make healthier food choices is a key issue for the prevention of cancer and other diseases. In many countries, authorities are considering implementing a simplified labelling system to reflect the nutritional quality of food products. However, although it would comply with the European Union labelling regulations, appending the Nutri-score on food product labels remains optional and, therefore, relies on voluntary uptake by food manufacturers (*Deschasaux et al*., 2018). Nonetheless, a unique nutritional labelling system for all EU countries is expected to be implemented in the future (*Deschasaux et al*., 2018).

## Conclusion

The domestic meat industry in Serbia needs to make special, focused efforts to optimize the production of meat products, since 82.5% of the retail meat products were classified as unhealthy by the Nutri-score method. In doing so, the specifics of the type of meat product could be taken into account for some products (crackling, bacon, dried meat products, fermented sausages and pâté) that are not intended to be consumed in amounts as large as 100 g per day. Nonetheless, it is necessary to determine the nutritional score for every food based on the 100 g or ml set amount to enable consumers to compare different foods and improve their own food choices.

For meat products that have potential for nutritional score optimization (meat preparations, meat dishes, canned minced meat, canned meat chunks, boiled and cooked sausages), producers are advised to pay special attention to reducing levels of the particularly burdensome nutritional parameters, i.e., sodium chloride/sodium, saturated fat and the overall energy balance.

# Nutritivni skor proizvoda od mesa na domaćem tržištu

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*A p s t r a k t: Nutritivni skor je pojednostavljeni sistem obeležavanja nutritivnog kvaliteta proizvoda od mesa, koji ih svrstava u pet kategorija sa odgovorajućom bojom i slovnom oznakom. Na ovaj način potrošači dobijaju dodatnu informaciju o nutritivnim svojstvima proizvoda od mesa koje kupuju, dok industrija dobija podsticaj da razvija kvalitetnije proizvode. Tokom dve godine ispitano je 310 uzoraka, od kojih je 82.5% klasifikovano kao ,,nezdravo" dok je samo 2.9% uzoraka koji su se odnosili na sveže i ustinjeno meso klasifikovani kao ,,zdravi". Nutritivni skor proizvoda od mesa je pokazao da 41.5% proizvoda od mesa imaju ocenu E, 41% ocenu D, 13% ocenu C, 1.6% ocenu B, dok je svega 2.9% dobilo ocenu A. Posebno opterećujući nutritivni parametar za proizvode od mesa je visok procenat soli natrijuma (77%), zatim prisustvo zasićenih masti (54%) i visok energetski bilans (15%). Cilj ovog rada je da utvrđivanjem nutritivnog skora proizvoda od mesa potrošačima na domaćem tržištu ukaže na značaj adekvatnog izbora proizvoda od mesa sa aspekta nutritivnog kvaliteta, dok sa druge strane takve zahteve predoči industriji mesa.*

***Ključne reči:*** *nutritivni skor, proizvodi od mesa, deklarisanje hrane, so, zdrava hrana.*

**Disclosure statement:** No potential conflict of interest was reported by authors.

**Acknowledgements:** This study was funded by grants TR 31083 and III 46009 from the Ministry of Education, Science and Technological Development, Republic of Serbia.

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Paper received: April 7th 2020.

Paper corrected: May 12th 2020.

Paper accepted: May 12th 2020.

**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 635.8:615.279*
*ID: 16241161*
*https://doi.org/10.18485/meattech.2020.61.1.5*

*Original Scientific Paper*

# Antioxidant activity of mushrooms *in vitro* and in frankfurters

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*A b s t r a c t: The antioxidant properties of Boletus edulis, Cantharellus cibarius and Craterellus cornicupioides decoctions and the effect of mushroom addition on the total phenolic content and the degree of secondary oxidative changes on lipids in frankfurters was studied. Moderate antioxidant activity was obtained by DPPH assay when mushroom decoctions were tested in vitro. Using the conjugated diene method, moderate antioxidant activity was achieved with Boletus edulis and Craterellus cornicupioides decoction, while with Cantharellus cibarius decoction, antioxidant activity was low. Constant amounts of phenolic acid were obtained in frankfurters fortified with Boletus edulis, while lipid oxidation on each tested day was several times less than in the control group of frankfurters, throughout two months of refrigerated storage. Generally, these mushrooms could be used as natural antioxidants to interfere with the chemical deterioration of food products and specifically, to extend the shelf life of cooked pork sausages.*

***Keywords:*** *Antioxidant, Natural extract, Shelf life, Mushroom, Phenolics.*

## Introduction

Emulsification technology for frankfurter-type sausage production has been used for several hundred years. Frankfurters are the most widespread type of emulsified meat product in the world (*Fernández-López et al.,* 2019). Due to volatility of the used meat, spices and other components, effects of high temperatures in thermal treatment and different storage conditions, cooked sausages are exposed to microbiological (*Sachindra et al.,* 2005), chemical and sensory degradation (*Hayes et al.,* 2011).

Lipid oxidation is recognized as the major problem producing negative effects on the quality and shelf life of meat products, causing oxidative off-flavours, discoloration and spoilage of meat and meat products (*Morrissey et al.,* 1998). Hence, there is presently increasing interest in the control of lipid oxidation in meat products by using antioxidant compounds from synthetic and natural sources (*Deda et al.,* 2007; Özvural & Vural, 2011). However, it is assumed that the existing synthetic antioxidants cause toxicity problems that negatively affect consumers' health, and therefore, usage of these compounds is limited in food (*Botterweck et al.,* 2000). A new trend in partially and totally substituting these synthetic antioxidants with antioxidants from natural sources received the most attention among consumers and meat processors (*Ahn et al.,* 2004; *Deda et al*., 2007; *Yılmaz et al.,* 2002). Thus, a need for recognizing alternative safe sources of natural antioxidants, specifically of plant origin, has considerably increased in recent years (Skerget et al., 2005).

Mushrooms can be an alternative, less processed and readily available source of natural antioxidants (*Djekic et al*., 2017a). Drying mushrooms is beneficial, as it concentrates mushroom nutrients such as heat-stable minerals, proteins and umami mixtures. They contain various polyphenolic compounds known as excellent antioxidants (*Djekic et al*., 2017b) due to their capability of scavenging free radicals by single-electron transfer (*Hirano et al*., 2001). Therefore, the objective of this study was to investigate the antioxidative effect of *Boletus edulis* (BE), *Cantharellus cibarius* (CaC) and *Craterellus cornicupioides* (CrC) *in vitro* and in frankfurters, in order to examine the potential antioxidant effect of the mushrooms on lipid oxidation during the refrigerated storage of frankfurters.

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## Materials and Methods

*Sample preparation*

In order to obtain a decoction, a mixture (1:10) of dry powdered mushroom and Milli-Q (MQ) water obtained from a Milli-Q water purification system (Merck, Darmstadt) was heated at 80°C, 1 h. The resulting decoction was subjected to all further analyses and also, together with a solid part, it was used as a component in making frankfurters.

*Antioxidant capacity of mushroom decoctions in vitro*

*DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay*

The method was performed according to *Vunduk et al.* (2015). Extract solutions were prepared in MQ water (Merck, Darmstadt).

*Lipid peroxidation*

The conjugated diene method according to *Lingnert et al.* (1979) was used.

*Preparation of frankfurters*

The decoctions of mushrooms were prepared as follows: 60 g of powder was added to 2 L of distilled water and heated at 80°C. The mixture was subjected to reflux for 60 min at 80°C. The decoctions obtained in this way were poured into a plastic container, cooled, and frozen to obtain ice. This was repeated in triplicate and added to frankfurters during production (T1). The same procedure was applied to the following batch (T2), with the exception that 120 g of powder was used in each individual batch. The control frankfurter formulation (C) was prepared with ice obtained from distilled water. All treatments were formulated to obtain 8 kg batter and each treatment was prepared in triplicate (Table 1).

To determine whether the mushroom decoctions released antioxidant activities in the frankfurters, the common spices such as onion, garlic, etc. were not added, and the sausages were not subjected to smoking, because these spices and smoke can also have antioxidant characteristics which could affect and mask the real effect of the added decoctions in the products. Fresh pork hams (*Musculus Biceps femoris*, *M. Semitendinosus* and *M. Semimembranosus*) and pork back fat at 48 h post-mortem were bought from a local abattoir. All connective tissue and visible fat were removed from the ham muscles. Lean meat and back fat were minced through an 8-mm plate using a meat grinder (Laska 82H, Austria). The meat was transferred to a bowl chopper (Müller EMS, Germany), and salt and polyphosphate were added. The meat was comminuted for 3 min at low speed to extract myofibrillar proteins until the temperature reached 6°C, when other ingredients were slowly added. The temperature of the mixture was not allowed to exceed 12°C (*Costa-Lima et al.*, 2014). After emulsification, meat batters were immediately stuffed into collagen casings (Edicas, Girona, Spain; approximately 22 mm diameter) using a stuffer. The frankfurters were cooked at 80°C in a smokehouse until the core temperature of 72°C was reached. The cooked frankfurters were cooled using a shower. Thereafter, the frankfurters were placed in vacuum bags (3 frankfurters/bag, all from an individual batch) (day 0). The vacuum bags containing frankfurters were then sealed with a tabletop vacuum machine, (MVS 35x, Minipack-Torre SpA, Italy) and stored at 1–4°C. All the experiments were conducted in the pilot meat processing plant at the Animal Source Food Technology Department of the Faculty of Agriculture, University of Belgrade.

**Table 1.** Formulae of the different types of frankfurters (expressed as % of the different ingredients in the formulae).

| | T1[1] | T2[1] | C[1] |
|---|---|---|---|
| Meat | 48% | 48% | 48% |
| Fat | 25% | 25% | 25% |
| Ice | – | – | 25% |
| Decoction 1 | 25% | – | – |
| Decoction 2 | – | 25% | – |
| Sodium nitrite | 1.7% | 1.7% | 1.7% |
| Polyphosphate | 0.3% | 0.3% | 0.3% |

**Legend:** [1] T1: concentration of 0.75 % mushroom in the batch. T2: concentration of 1.5 % mushroom in the batch. C: control, ice instead of mushroom decoction.

*Total phenolic content*

Amounts of cooked frankfurters (5 g) were homogenized and extracted with 25 mL of ethanol (96%). The extraction was carried out in an Ultraturax at 15000 rpm for 2 min. The resulting extract was filtered through a 1–2 μm filter paper. After filtration, 1 mL of the extract was added to a cuvette, followed by Folin-Ciocalteu reagent (0.5 mL) and saturated sodium carbonate solution (1 mL). After 1 h, the blue dye formed was measured at a wavelength of 725 nm against a blank using a Jenway 6300 spectrophotometer (Jenway, Felsted, United Kingdom). The phenol content was calculated based on the calibration curve (concentration-dependent absorbance function) of the standard solution of gallic acid. The result was expressed as milligram equivalents of gallic acid per kilogram of the sample – mg GAE $kg^{-1}$ (*Naveena et al.*, 2013). As a result, the arithmetic means of the phenol content were determined in three finely ground baked frankfurters from each of the sausage batches examined.

*TBARs determination*

TBARs (2-thiobarbituric acid reactive substances) test was accomplished using the method of *Bostoglou et al.* (1994), with the following alterations. The total volume of trichloroacetic acid (TCA) was added to the sample, and extraction was performed in an ultrasonic bath XUB 12 (Grant Instruments, Cambridge, UK) (Sojic et al., 2015). A Jenway spectrophotometer 6300 (Jenway, Felsted, United Kingdom) was used to measure absorbances. TBARs analyses were performed on three frankfurters from each batch, and results were expressed as mean milligrams of malondialdehyde (MDA) per kilogram of frankfurter.

*Statistical analysis*

The data for phenolic content and TBARs analyses were analyzed using the mixed split plot model ANOVA procedure considering 'storage day' and 'treatment' as independent variables. Mean differences between groups were tested using Bonferroni's *post hoc* test operating at a 5% level of significance. All statistical analyses were carried out using SPSS for Windows (SPSS 23.0, Chicago, IL, USA).

The data obtained from the antioxidant analyses of mushroom decoctions *in vitro* were processed using one-way analysis of variance (ANOVA). Tukey's HSD *post hoc* test was used to distinguish statistical differences between the sausages and storage ($p<0.05$).

## Results and Discussion

*Scavenging capacity as measured by DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay*

The DPPH assay, based on the measurement of the scavenging capacity of antioxidants towards the stable radical, DPPH, is one of the most common techniques for the determination of antioxidant capacity (*Abdullah et al.*, 2012). Concentration-dependent scavenging activity was observed in all frankfurters (Table 2). Compared to the positive control used (L-ascorbic acid), mushrooms tested

**Table 2.** Scavenging ability of the mushroom decoctions and the commercial antioxidant, L-ascorbic acid as measured by DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay.

| Concentration (mg $mL^{-1}$) | BE[1] | CaC[1] | CrC[1] | AA[1] |
|---|---|---|---|---|
| 0.625 | $21.1\pm0.95^{a,A}$ | $12.72\pm10.02^{ab,A}$ | $5.36\pm4.38^{b,A}$ | $81.33\pm1.11^{c,A}$ |
| 1.25 | $35.42\pm7.13^{a,AB}$ | $13.43\pm2.95^{b,A}$ | $16.48\pm3.85^{ab,A,B}$ | $83.65\pm0.09^{c,B}$ |
| 2.5 | $32.96\pm11.56^{a,A}$ | $20.57\pm1.85^{ab,A,B}$ | $14.63\pm6.91^{b,A}$ | $83.38\pm0.15^{c,B}$ |
| 5 | $43.62\pm15.97^{a,AB}$ | $26.08\pm9.15^{a,B}$ | $24.7\pm6.02^{a,A,B}$ | $84.38\pm0.26^{b,B}$ |
| 10 | $57.55\pm7.19^{a,B}$ | $31.2\pm1.1^{b,A}$ | $29.46\pm1.11^{b,B}$ | $81.07\pm0.49^{c,A}$ |
| $EC_{50}$ | 4.46 | 7.41 | 8.65 | – |

**Legend:** [1]Abbreviations: BE – *Boletus edulis*; CaC – *Cantharellus cibarius*; CrC – *Craterellus cornicupioides*; AA – L-ascorbic acid; $EC_{50}$ value (mg $mL^{-1}$) is the effective concentration at which DPPH radicals were scavenged by 50%. Notes: Values are mean±standard deviation. Means in the same column with different capital letters and means in the same row with different lowercase letters are significantly different ($p<0.05$)

by this assay had significantly lower values for each concentration.

The scavenging activity of mushroom extracts towards DPPH free radicals can also be expressed in terms of $EC_{50}$. The $EC_{50}$ value (mg $mL^{-1}$) is the effective concentration at which 50% of the DPPH radicals were scavenged and was obtained by interpolation from the linear regression analysis. $EC_{50}$ values for BE, CaC and CrC were 4.46, 7.41 and 8.65, respectively. A lower $EC_{50}$ value corresponds to a higher antioxidant activity of the mushroom extract. *Puttaraju et al.* (2006) reported $EC_{50}$ values for hot water extract of BE and CaC to be 1.30 and 6.40, respectively.

We wanted to test exactly this method (decoction), due to its convenience (easy, fast, simple and cost-effective) that would mean it could later easily find its way to industrial application. Concerning CrC, the extract used in this study (8.65 mg $mL^{-1}$) had a higher antioxidant effect than the hot aqueous extract of the same mushroom, reported in the study of *Liu et al.* (2012) (26.37 mg $mL^{-1}$). The ability of hot water extract to quench free radicals has been reported by many researchers. *Chirinang and Intarapichet* (2009) reported strong antioxidant activity of mature and baby Ling chih (*Ganoderma tsugae* Murrill*)*, with low $EC_{50}$ of 0.30 and 0.40 mg $mL^{-1}$, respectively. The same authors reported moderate antioxidant activity with the same extraction technique for *Pleurotus ostreatus* ($EC_{50}$ = 11.56 mg $mL^{-1}$) and for *P. sajor-caju* ($EC_{50}$ = 13.38 mg $mL^{-1}$). In the study of Öztürk et al. (2007), *Agaricus blazei*, *Agrocybe cylindracea* and *B. edulis* displayed moderate DPPH scavenging activities with $EC_{50}$ of 13.75, 26.98 and 15.78 mg $mL^{-1}$, respectively. In total, the decoctions of the three mushrooms tested by DPPH assay showed moderate scavenging ability in comparison to the literature reports on other mushrooms.

*Lipid peroxidation as measured by the conjugated diene method*

Using the conjugated diene method, at the concentration of 10 mg $mL^{-1}$, antioxidant activities of the decoctions were 53.94±5.27, 22.36±1.34 and 68.75±0.33% for BE, CaC and CrC, respectively (Table 3). Also, antioxidant activity obtained by these three mushrooms was dose-dependent, reaching their maxima at 10 mg $mL^{-1}$. In comparison to the positive control (L-ascorbic acid), our values were significantly lower at each tested concentration.

At the same concentration, measured with the same method, *Mau et al.* (2005) reported antioxidant activity about 60% for Ling chih (*Ganoderma tsugae* Murrill) mushroom. These values correspond to the values obtained in our study for BE and CrC, while the antioxidant activity we obtained from CaC was significantly lower (Table 3). Similar results for antioxidant activity at the same tested concentration for *Pleurotus citrinopileatus* mushroom were reported by *Lee et al.* (2007). In their study, *Tsai et al.* (2007) reported considerably higher values (66.3%, 83.0%, and 85.7% for *Agaricus blazei*, *Agrocybe cylindracea*, and *B. edulis*, respectively) in comparison to our results at the same tested concentration (5 mg $mL^{-1}$).

The conjugated-diene method is based on the ability of the tested compound to slow down the oxidation of conjugated dienes, which can be formed only from polyunsaturated fatty acids (*Huang et al.*, 2005). Since linoleic acid is used as a substrate in

**Table 3**. Ability of mushroom decoctions and the commercial antioxidant, L-ascorbic acid, to prevent the peroxidation of linoleic acid.

| Concentration of active compound (mg $mL^{-1}$) | BE[1] | CaC[1] | CrC[1] | AA[1] |
|---|---|---|---|---|
| 0.1 | $0±0^{a,A}$ | $0±0^{a,A}$ | $14.14±2.31^{b,A}$ | $78.33±0.91^{c,A}$ |
| 1 | $0±0^{a,A}$ | $0±0^{a,A}$ | $27.52±0.18^{b,B}$ | $79.5±0.75^{c,A,B}$ |
| 2.5 | $15.63±1.06^{a,B}$ | $0±0^{b,A}$ | $44.07±1.32^{c,C}$ | $79.6±0.9^{d,A,B}$ |
| 5 | $40.79±1.97^{a,C}$ | $11.75±1.98^{b,B}$ | $62.5±1.32^{c,D}$ | $80.84±0.72^{d,B}$ |
| 10 | $53.94±5.27^{a,D}$ | $22.36±1.34^{b,C}$ | $68.75±0.33^{c,E}$ | $82.73±0.8^{d,C}$ |

**Legend:** [1]Abbreviations: BE – *Boletus edulis*; CaC – *Cantharellus cibarius*; CrC – *Craterellus cornicupioides*; AA – L-ascorbic acid. Notes: Values are mean±standard deviation. Means in the same column with different capital letters and means in the same row with different lowercase letters are significantly different ($p<0.05$)

this assay, it is not completely the same as a biological system and forms only one type of conjugated diene. Hence, this method is not enough for the complete evaluation of the ability of an antioxidant substance to act preventively in complex systems like food. Thus, when testing the antioxidant properties of the final frankfurter products, we also used another method, more specific to meat products.

*Total phenolic content of frankfurters during cold storage*

The main compounds responsible for the antioxidant activity of mushrooms are phenolics (*Elmastas et al.*, 2007). Our results showed frankfurters fortified with BE mushroom contained the highest amount of phenolics, while CaC and CrC frankfurters contained significantly lower levels of phenolics, on each day of examination during storage (Figure 1). Previously, researchers confirmed the relationship between total phenolic compounds and antioxidant activity was linear (*Annegowda et al.*, 2013; *Isabelle et al.*, 2010). Also, phenolic compounds in our frankfurters with BE mushroom remained almost unchanged during the storage, which agrees with the findings of *Ribas-Agusti et al.* (2014) and *Van Ba et al.* (2016). On the other hand, lower phenolic concentrations throughout the storage period were obtained for frankfurters prepared with extracts obtained from CaC and CrC. *Palacios et al.* (2011) reported that chlorogenic, *p*-coumaric, homogentisic and protocatechuic acids were found in *B. edulis,* but not in *Cantharellus cibarius* or *Craterellus cornicupioides*. Also, the same researchers reported higher amounts of gallic, gentistic and *p*-hydroxybenzoic acids in BE than in CaC and CrC extracts, which could explain the persistence during storage of the antioxidant compounds in our frankfurters prepared with BE decoction.

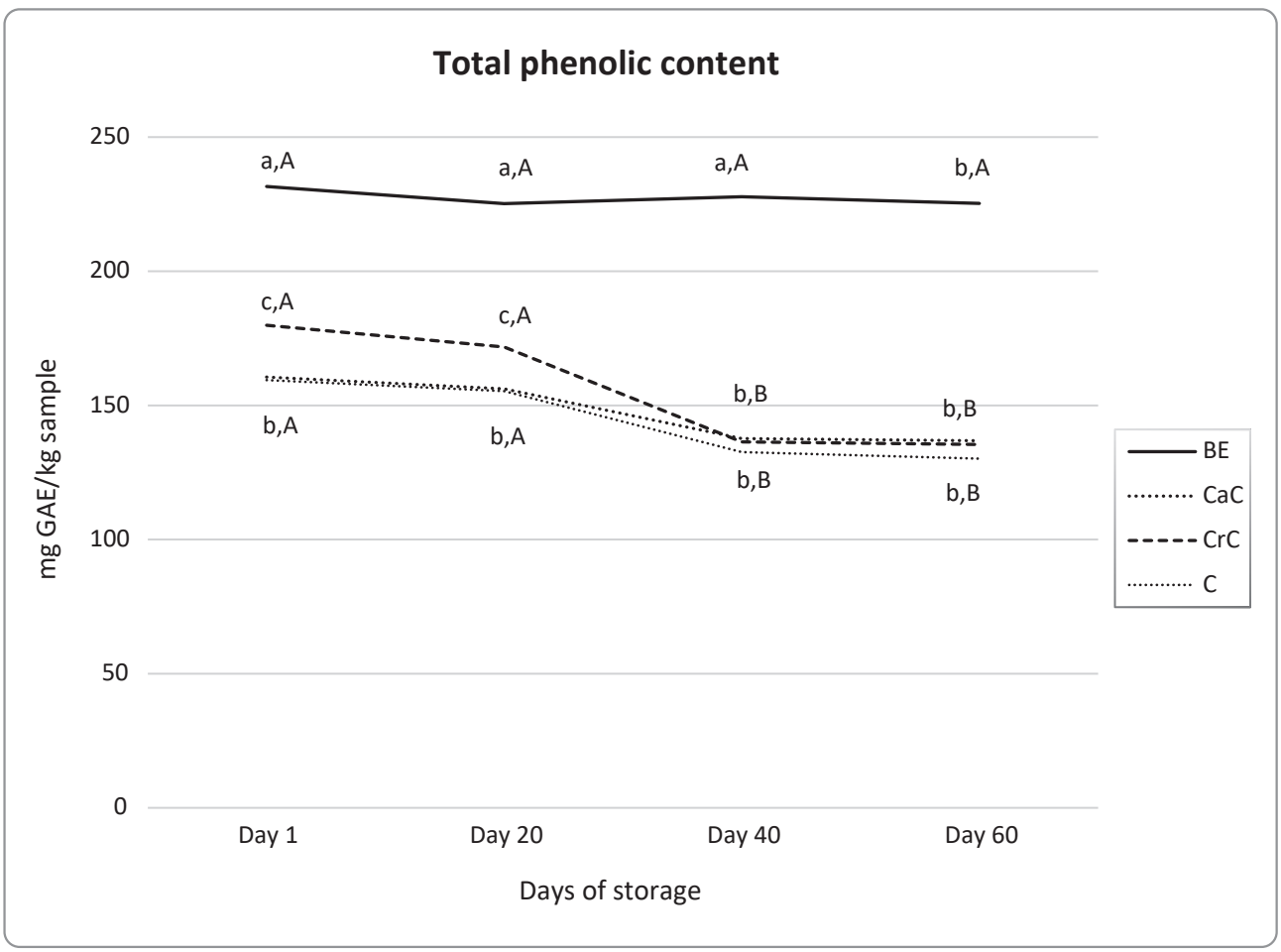


**Figure 1.** Total phenolic content in frankfurters, expressed as mg GAE/kg of sample.

**Legend:** [1]Abbreviations: BE – *Boletus edulis*; CaC – *Cantharellus cibarius*; CrC – *Craterellus cornicupioides*; C – control. Values with different lowercase letters (a-c) in the same column (for the different treatments) differ significantly ($p<0.05$). Values with different uppercase letters (A-B) on the same line (for the same treatment at different storage time) differ significantly ($p<0.05$).

**Table 4.** Thiobarbituric acid reactive substances (TBARs) values of frankfurters formulated with different mushroom decoctions during refrigerated storage.

| Day of Storage | BE[1] | CaC[1] | CrC[1] | C[1] |
|---|---|---|---|---|
| Day 1 | $0.22\pm0.02^{a,A}$ | $0.34\pm0.04^{b,A}$ | $0.14\pm0.02^{c,A}$ | $0.56\pm0.02^{d,A}$ |
| Day 10 | $0.33\pm0.01^{a,B}$ | $0.45\pm0.01^{b,B}$ | $0.31\pm0.02^{a,B}$ | $0.71\pm0.02^{c,B}$ |
| Day 20 | $0.20\pm0.01^{a,A,C}$ | $0.46\pm0.02^{b,B}$ | $0.42\pm0.03^{b,C}$ | $0.59\pm0.02^{c,A,C}$ |
| Day 30 | $0.17\pm0.01^{a,C,D}$ | $0.19\pm0.01^{a,C}$ | $0.17\pm0.02^{a,A}$ | $0.63\pm0.01^{b,C}$ |
| Day 40 | $0.16\pm0.01^{a,D}$ | $0.15\pm0.01^{a,D}$ | $0.11\pm0.01^{a,D}$ | $0.60\pm0.04^{b,C}$ |
| Day 50 | $0.12\pm0.01^{a,E}$ | $0.08\pm0.01^{b,E}$ | $0.09\pm0.01^{ab,D}$ | $0.37\pm0.01^{c,D}$ |
| Day 60 | $0.04\pm0.02^{ab,F}$ | $0.07\pm0.01^{a,E}$ | $0.03\pm0.01^{b,E}$ | $0.16\pm0.01^{c,E}$ |

**Legend:** [1]Abbreviations: BE – *Boletus edulis*; CaC – *Cantharellus cibarius*; CrC – *Craterellus cornicupioides*; C – control. Notes: Values are mean±standard deviation. Means in the same column with different capital letters (A-F) and means in the same row with different lowercase letters (a-c) are significantly different ($p<0.05$)

*Oxidative changes in frankfurters during cold storage as measured by TBARs assay*

The TBARs assay is one of the most frequent methods used for determining the degree of secondary oxidative changes on lipids in meat and meat products (Sojic et al., 2017). On storage day 1, TBARs values for frankfurters with mushrooms were significantly lower in comparison to the control frankfurters (Table 4). This was most likely the consequence of the presence of phenolics in the mushrooms, mixtures that are mainly responsible for the antioxidant activity of many plants (*Elmastas et al.*, 2007). Our TBARs assay results were in accordance with the literature data for a similar type of meat product (*Hwang et al.*, 2015). Additionally, on all days of examination, the incorporation of mushroom decoctions in T1 and T2 frankfurters resulted in significantly lower TBARs values than was found in control frankfurters without mushroom decoction. Relative to the control group, frankfurters with the added mushroom decoctions had up to several times lower TBARs values in some cases. It should be mentioned that all TBARs values were less than 1, the value that *Ockerman* (1985) found to be the limit for the formation of rancidity in meat products.

Throughout frankfurter storage, trends of increasing and then decreasing TBARs values were noticed. We suggest the decline in TBARs values could be attributed to the creation of MDA, an intermediate product; until a certain point, the rate of MDA creation was higher than the rate of its disappearance, and thereafter, the reverse was true. Thus, the disappearance rate overshot the rate of creation, and hence, TBARs values declined (*Bhattacharya et al.*, 1988). Similarly, according to *Jamora and Rhee* (2002), MDA formed during meat product storage might be subjected to intermolecular reactions (polymerization) and reactions with other constituents, especially amino acids/proteins. Since we used mushroom decoction, which is a complex of polysaccharides, proteins, peptides, and free amino acids, it is very likely that polymerization occurred. Therefore, the MDA disappearance (loss) rate during storage can be higher than the rate of formation by lipid oxidation.

## Conclusion

The mushroom decoctions used in this study expressed measurable antioxidative effects in the prepared frankfurters. We believe mushroom decoctions show potential and should be considered as a natural replacement of the commercial antioxidants in this kind of meat product. Further research is needed to evaluate the overall quality of frankfurters with added mushroom decoction, to be sure that mushroom addition does not have a detrimental effect on the quality and safety parameters of the final product.

# Antioksidativna aktivnost gljiva in vitro i u frankfurterima

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*A p s t r a k t: Ispitivane su antioksidativne karakteristike dekokta vrganja (Boletus edulis), lisičarke (Cantharellus cibarius) i crne trube (Craterellus cornicupioides) i uticaj dodatka gljiva na ukupni sadržaj fenolnih komponenti i stepen sekundarnih oksidativnih promena na mastima u frankfurterima. Umerena antioksidaivna aktivnost dobijena je korišćenjem DPPH metode prilikom testiranja dekokta gljiva in vitro. Korišćenjem konjugen dienske metode, umerena antioksidativna aktivnost postignuta je sa dekoktima vrganja (Boletus edulis)i crne trube (Craterellus cornicupioides), dok je sa lisičarkom (Cantharellus cibarius) taj efekat bio slab. Konstantne količine fenolnih kiselina dobijene su u frankfurterima sa dodatkom vrganja (Boletus edulis), dok je oksidacija lipida prilikom svakog testiranog dana bila nekoliko puta manja u poređenju sa kontrolnom grupom frakfurtera, tokom dva meseca skladištenja u frižideru. Generalno, ove gljive mogu biti korišćene kao prirodni antioksidansi kako bi ometali hemijske produkte kvara i produžili rok trajanja kuvanih svinjskih kobasica.*

***Ključne reči:*** *Antioksidanti, prirodni ekstrakti, održivost, vrganji, fenoli.*

**Disclosure statement:** No potential conflict of interest was reported by the authors.

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Paper received: March 24th 2020.
Paper accepted: April 23th 2020.

**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 638.162:615.099*
*ID: 16251145*
*https://doi.org/10.18485/meattech.2020.61.1.6*

*Original scientific paper*

# Risk assessment of toxic elements in acacia honey

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*A b s t r a c t: The element concentrations (As, Cu, Zn, Fe, Cd and Pb) of 25 acacia honeys from Serbia were analysed using inductively coupled plasma mass spectrometry (ICP-MS). Concerning the toxic element concentrations, all tested honeys met Serbian legislation. Zinc was the major element, ranging between 0.37 mg $kg^{-1}$ and 3.95 mg $kg^{-1}$. Positive and significant correlations were found between Fe and Cu (r=0.567). This study showed the Serbian honey examined was good quality and met safety criteria concerning concentrations of As, Cu, Zn, Fe, Cd and Pb.*

***Keywords:*** *acacia honey, Serbia, consumption, food safety.*

## Introduction

Honey bees and bee products are significant bioindicators of environmental pollution and heavy metals, since bees visit areas of up to 7 $km^2$ (*Spiric et al*., 2019; *Pisani et al*., 2008; *Rashed et al*., 2009; *García-Valcárcel et al*., 2016; *Matin et al*., 2016; *Ciric et al*., 2018). During harvest time, bees are exposed to different sources of contaminants through their pollen and nectar that contains heavy metals of natural and/or anthropogenic origin (*Chauzat et al*., 2009). Toxic elements are bioaccumulative, and they can be measured in honey bee tissues and bee products (*Bogdanov*, 2006; *Oroian et al*., 2016; *Gomes et al*., 2010). On the other hand, many elements have a number of important functions in chemical processes and their fingerprints can be used to determine the botanical and geographic origin of honeys (*Erbilir* and *Erdoğrul*, 2005). Elements such as Cu, Fe, Mn, Co, Ni and Zn are essential elements that have roles in cell metabolism, but in excess amounts, they are harmful. The toxic elements that have negative impact are Pb, Cd, Cr and Hg. Pb and Cd are toxic heavy metals and are, thus, the most frequently studied (*Bogdanov et al*., 2006). Pb originating mainly from motor traffic can contaminate air and then directly nectar and honeydew. Generally, Pb is not transported by plants (*Bogdanov et al*., 2006). Pb contamination is expected to diminish, due to the increased world-wide use of car-engine catalysts. Cd originating from the metal industry and incinerators is transported from the soil to plants and can then contaminate nectar and honeydew (*Bogdanov et al*., 2006). Only a small portion of Cd might reach honey by air, mainly in the vicinity of incinerators. Also, Cd and Pb are environmental pollutants and are used as honey quality indicators.

Serbian honey production, according to *Ivanovic* et al. (2015), amounts to approximately 4,200 tons in recent years and could potentially be exported to the EU. The most common honeys produced in Serbia are the monoflorals, acacia (*Robinia pseudoacacia*), linden (*Tiliaeuropea*) and sunflower (*Helianthus annuus*), and multifloral honey (*Lazarevic et al*., 2012; *Matovic et al*., 2018). So far, existing scientific data on toxic element (As, Cu, Zn, Fe, Cd and Pb) concentrations in honeys from Serbia is very limited. In the present study, the composition of six elements was determined in acacia honey from Serbia. The objectives of this study were to: I) analyse toxic element concentrations and; II) determine potential correlations between some toxic element concentrations in acacia honey.

## Materials and methods

### *Honey samples*

A total of 25 acacia honeys (collected from different parts of Serbia during the 2018 harvest season) were examined. The botanical origin of the

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**Table 1.** Quality control of analysis

| Elements | Limit of detection | Limit of quantification | Method repeatability/ precision as RSD (%) | Certified value[a] | Analysed value[b] |
|---|---|---|---|---|---|
| As | (μg kg$^{-1}$) 1.2 | (μg kg$^{-1}$) 4 | 3.57 | (μg kg$^{-1}$) 19.3±1.4 | (μg kg$^{-1}$) 20.5±1.1 |
| Cu | (mg kg$^{-1}$) 0.022 | (mg kg$^{-1}$) 0.066 | 6.26 | (mg kg$^{-1}$) 275.2±4.6 | (mg kg$^{-1}$) 271.9±5.7 |
| Zn | (mg kg$^{-1}$) 0.124 | (mg kg$^{-1}$) 0.372 | 10.52 | (mg kg$^{-1}$) 181.1±1.0 | (mg kg$^{-1}$) 180.9±1.8 |
| Fe | (mg kg$^{-1}$) 0.08 | (mg kg$^{-1}$) 0.23 | 4.71 | (mg kg$^{-1}$) 197.94±0.65 | (mg kg$^{-1}$) 197.43±5.21 |
| Cd | (μg kg$^{-1}$) 0.4 | (μg kg$^{-1}$) 1 | 8.99 | (μg kg$^{-1}$) 97±1.4 | (μg kg$^{-1}$) 97.9±2.6 |
| Pb | (μg kg$^{-1}$) 2 | (μg kg$^{-1}$) 3.8 | 3.65 | (μg kg$^{-1}$) 62.8±1.0 | (μg kg$^{-1}$) 63.3±2.6 |

**Legend:** [a]Certified value as given by the manufacturer; [b]Data are mean±standard deviation.

honeys was established by information provided by beekeepers. Honeys (500 g) were stored in glass containers at 4–8°C until analysis.

*Element concentration analyses*

Approximately 0.5 g of homogenized honey was transferred into a Teflon vessel with 5 ml nitric acid (67% Trace Metal Grade, Fisher Scientific, Loughborough, UK) and 1.5 ml hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MO, USA) for microwave digestion. The microwave (Start D, Milestone, Sorisole, Italy) programme consisted of three steps: 5 min from RT to 180°C, 10 min hold at 180°C, and 20 min cooling. After cooling, the digested honeys were quantitatively transferred into disposable flasks and diluted up to 100 ml with deionized water produced by a water purification system (Purelab DV35, ELGA, High Wycombe, Buckinghamshire, UK). Analysis of the following six elements, As, Cu, Zn, Fe, Cd and Pb, was performed by inductively coupled plasma mass spectrometry (ICP-MS) (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany).

Adjustment of physical and electronic parameters was performed before determining the elements, using calibration solution (Thermo Scientific Tune B). The calibration curve consisted of five points in two ranges (including zero). Cadmium and As were measured in the range 0.2–2.0 μg kg$^{-1}$ and Pb, Fe, Cu and Zn in the range 2.0–20.0 μg kg$^{-1}$. Multielemental internal standard was introduced into the ICP-MS during the measurements. Data analysis software automatically made corrections comparing internal standards. Quality control was performed using certified reference material (CRM) NIST SRM 1577c (Table 1).

*Statistical analysis*

Statistical analysis was performed using the GraphPad Prism version 7.00 software. The concentrations of elements in different honey types were expressed as the minimum and maximum, mean±standard error (SE) and were subjected to analysis of variance (one-way ANOVA). The parameters were analysed using the Student's t-test at the probability of 0.05.

## Results and discussion

Toxic element (As, Cu, Zn, Fe, Cd and Pb) concentrations in acacia honey from Serbia are presented in Table 2. The value presented for each element is the average concentration observed. The values of the toxic metals concentrations were compared with those established by the European Union (*European Union*, 2006) and Serbian legislation (*Official Gazette*, 2018). The As concentration was 0.004 mg kg$^{-1}$ in the acacia honey from Serbia. The mean value of As content in honey samples was different in previous studies in Serbia (*Spiric et al*., 2018), Italy (*Pisani et al*., 2008), Slovenia (*Golob et al*., 2005) and New Zealand (*Vanhanen et al*., 2011).

**Table 2.** Mean±standard error of the mean, minimum and maximum levels of toxic elements in 25 acacia honeys from Serbia (mg kg$^{-1}$)

| Parameter | Mean | SEM | Min | Max |
|---|---|---|---|---|
| As | 0.004 | – | 0.004 | 0.004 |
| Cu | 0.147 | 0.0175 | 0.070 | 0.300 |
| Zn | 1.57 | 0.2398 | 0.370 | 3.950 |
| Fe | 1.30 | 0.0989 | 0.600 | 2.040 |
| Cd | 0.003 | 0.00001 | 0.001 | 0.004 |
| Pb | 0.004 | 0.00001 | 0.004 | 0.004 |

As (arsenic) is used industrially as an alloying agent, as well as in the processing of glass, pigments, textiles, paper, metal adhesives, wood preservatives, and ammunition. As is also used in the hide tanning process and, to a limited extent, in pesticides, feed additives, and pharmaceuticals. According to Serbian legislation, the maximum residue limit for As is 0.500 mg kg$^{-1}$, and all tested samples of acacia honey met this legislation.

The Cu content ranged between 0.070 mg kg$^{-1}$ and 0.300 mg kg$^{-1}$ with an average concentration of 0.15 mg kg$^{-1}$. In another study of Serbian honey, the Cu concentration ranged between 94.14±41.29 ppb (µg kg$^{-1}$) (acacia) and 737.1±470.1 ppb (honeydew) in the 84 tested honeys (*Spirić et al*., 2018). The Cu content among honey from different countries ranged widely: 0.25 mg kg$^{-1}$ in the case of New Zealand honeys (*Vanhanen et al*., 2011), around 900 µg kg$^{-1}$ in the case of Italian honeys (*Pisani et al*., 2008) 0.25–1.10 mg kg$^{-1}$ in the case of honey from Turkey (*Tuzen et al*., 2005) and 3.22 mg kg$^{-1}$ in the case of Slovenian honeys (*Golob et al.*, 2005). Cu can be present in honey due to the copper fungicides used in agriculture. According to Serbian legislation (*Official Gazette*, 2018), honey should not contain more than 1 mg kg$^{-1}$ of Cu. The Cu content ranged between 0.070 mg kg$^{-1}$ and 0.300 mg kg$^{-1}$.

The Zn content in the acacia honeys ranged between 0.37 mg kg$^{-1}$ and 3.95 mg kg$^{-1}$. Other literature data showed significant differences in element concentrations between regions in countries or between different honeys in the same regions (*Silva et al*., 2009). Any increase of Zn indicates that honey is kept in inadequate packaging or was centrifuged in a galvanized centrifuge (*Jevtic et al.*, 2012). All the investigated acacia honeys met the requirements set by Serbian regulation (*Official Gazette*, 2018), which require that the Zn content not exceed 10 mg kg$^{-1}$.

Fe was the most abundant analysed metal in the examined honeys. The Fe content ranged between 0.600 mg kg$^{-1}$ and 2.040 mg kg$^{-1}$, with an average concentration of 1.30 mg kg$^{-1}$. The Fe content was in the same range as was reported in honeys from Turkey (1.8–10.2 mg kg$^{-1}$) (*Tuzen et al*., 2005) and another study of acacia honeys from Serbia (1.19 mg kg$^{-1}$) (*Spiric et al*., 2018).

The Cd content ranged between 0.001 mg kg$^{-1}$ and 0.004 mg kg$^{-1}$ in acacia honeys from Serbia, with a mean value of 0.003 mg kg$^{-1}$. The Cd content in honey differs from one country to another; in New

**Table 3.** Correlation between toxic elements in acacia honey

| | As | Cu | Zn | Fe | Cd | Pb |
|---|---|---|---|---|---|---|
| As | ns | ns | ns | ns | ns | ns |
| Cu | ns | ns | ns | 0.567* | ns | ns |
| Zn | ns | ns | ns | ns | ns | ns |
| Fe | ns | 0.567* | ns | ns | ns | ns |
| Cd | ns | ns | ns | ns | ns | ns |
| Pb | ns | ns | ns | ns | ns | ns |

**Legend:** *$p<0.05$; ns — not significant, $p>0.05$

Zealand it is 149.0 μg $kg^{-1}$ (*Vanhanen et al*., 2011), in Italy, 305 μg $kg^{-1}$ (*Pisani et al*., 2008), in Turkey it ranges between 0.9–17.9 μg $kg^{-1}$ (*Tuzen et al.*, 2005) and in Poland it is 15.0 μg $kg^{-1}$ (*Przybyłowski et al*., 2006).

The mean Pb content of our acacia honey was 0.004 mg $kg^{-1}$. Similar results were presented in the study by *Spiric et al*. (2019). The contamination levels of the toxic elements Pb and Cd measured in our study were low and the honeys were safe.

Many previously studies found correlations between toxic element concentrations in honey. Table 3 shows the correlations between toxic element concentrations in the Serbian acacia honeys. There were significant correlations (individual significance level is 0.05) only between the levels of Fe and Cu (r=567) in the honeys. Other toxic elements did not correlate significantly (p>0.05).

## Conclusion

The toxic element concentrations (As, Cu, Zn, Fe, Cd and Pb) in acacia honey from Serbia were examined, and the levels of Fe and Cu correlated. The Serbian honey was of good quality according to national regulation (*Official Gazette*, 2018). Also, Zn was the major element in the tested honeys, which is in agreement with other honeys around the world.

# Procena rizika toksičnih elemenata u bagremovom medu

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*A p s t r a k t: Koncentracije elemenata (As, Cu, Zn, Fe, Cd i Pb) u 25 uzoraka bagremovog meda iz Srbije analizirane su primenom ICP-MS. Koncentracija toksičnih elemenata u svim ispitivanih uzorcima meda bila je u skladu sa nacionalnim propisima. Cink je bio glavni element koji se kretao između 0,37 mg/kg i 3,95 mg/kg. Utvrđene su pozitivne i značajne korelacije između sadržaja Fe i Cu (r = 0,567). Ova studija je pokazala da je bagremov med iz Srbije bio dobrog kvaliteta i ispunio bezbednosne kriterijume za koncentracije As, Cu, Zn, Fe, Cd i Pb.*

***Ključne reči:*** *bagremov med, Srbija, konzumiranje, bezbednost hrane.*

**Disclosure statement:** No potential conflict of interest was reported by authors.

**Acknowledgments:** This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, through the funding of the Projects: Selected biological hazards to the safety/quality of food of animal origin and the control measures from farm to consumer (TR 31034), and; Improvement and development of hygienic and technological procedures in the production of foodstuffs of animal origin in order to obtain quality and safe products that are competitive on the world market (III 46009).

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Paper received: 27.11.2019.

Paper corrected:.3.12.2019.

Paper accepted: 3.12.2019.

**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 612.39-056.43*
*ID: 16207369*
*https://doi.org/10.18485/meattech.2020.61.1.7*

*Original Scientific Paper*

# Control of nutritive allergens in a hospitality kitchen

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*A b s t r a c t: Provision of hospitality services is a complex operation from the aspect of safety of employees and consumers, which makes necessary the introduction of the safety system, hazard analysis of critical control points (HACCP). In order to get a safe gastronomic product in terms of nutritive allergens, in addition to analysis of ingredients and finished product, validated cleaning protocols in hospitality kitchens are required as a prerequisite for successful risk management and hazard analysis for allergens. The application of regular allergen control enables food business operators to implement appropriate cleaning and sanitation protocols to reduce the risk of cross-contamination with allergens. The aim of this study was to highlight the significance of applying validated regimes for cleaning and control finished product, in order to define control measurements for the presence of nutritive allergens. This contributes to good hygienic practice (GHP) and good manufacturing practice (GMP) in hospitality facilities.*

***Keywords:*** *nutritive allergens, cleaning protocol, allergen control, hospitality.*

## Introduction

Under current European hygiene legislation (*European Commission*, 2008*;* 2009), food businesses are obliged to develop and implement food safety management systems (FSMS) including prerequisite programme (PRP) activities and hazard analysis and critical control point (HACCP) principles. This requirement is especially challenging for small food retail establishments, where a lack of expertise and other resources can limit the development and implementation of effective FSMS (*EFSA,* 2017). Therefore, the issue of food (gastronomic products/meals and beverages are hereafter termed food in this study) safety in hospitality is a complex field, where application of FSMS and, sometimes, simplified PRP activities is necessary in order to protect the consumer. PRPs are preventive actions and conditions which should be performed before and during HACCP implementation, and they are crucial for food safety.

Allergies and intolerances to food ingredients are a safety risk widely considered in the food industry. To prevent or minimize contamination and/or cross-contamination with allergens in hospitality businesses, all aspects of the processes used must be properly controlled. Food hygiene and safety is the result of the implementation of PRPs and procedures based on HACCP principles. In the case of declared allergens, their presence is due to their use as a raw material, ingredient or component in a given food product. In this case, their presence must be declared on prepacked foods or in the case of non-prepacked foods, this information should be given to consumers. The latter communication is possible using notices in the shop, restaurants, web-page information, etc. (*European Commission*, 2011; *Official Gazette of RS*, 2016*;* 2018).

However, the main danger to allergic or sensitive consumers is the presence of undeclared allergens mainly in raw materials or ingredients, and cross-contamination during storage, processing, distribution and between different products. Cross-contamination with, for example, an allergenic ingredient can result in the presence of traces of allergens in food. In most circumstances, the food producer is unaware of the presence of the allergen (*Cucu et al.,* 2013), and a preventive 'may contain' notice is not always applied (*EFSA,* 2017).

## Hygienic design

The production area, professional equipment and tools are, besides the human factor, among the most common causes of food cross-contamination in

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**Table 1.** The effect of detergents and water on common food residues (*Nikoleiski,* 2015*)*.

| Residue | Example | Reaction with water | Detergent |
|---|---|---|---|
| **Carbohydrate** | Sugar, starch | Soluble in water | Mild alkaline |
| **Protein** | Milk proteins or egg proteins | Some are soluble in water | Chlorinated or strong alkaline with peroxide |
| **Fat** | Oil | Emulsifies with water | Alkaline |
| **Non-organic material** | Salt | Soluble in water | Alkaline acid or phosphates |

hospitality facilities. It is desirable that production areas in a facility have a physically separated area for the production of food products that are without allergens. If there are not such conditions, the HACCP plan must precisely define procedures for cleaning and sanitation of equipment and tools used directly in the process of allergen-free food products, and procedures for serving this type of product (*Popov-Raljic et al.,* 2017). Hygienic design of food premises is basically conditioned by the EU directive (*Commission Directive*, 2006), which prescribes specific requirements for machines that come into contact with food. Also, in Serbia, the rulebook on conditions and manner of conducting hospitality business operations, manner of providing hospitality services, classification of hospitality facilities and minimum technical conditions for constructing and equipping hospitality facilities (*Official Gazette of RS,* 2016) prescribes requirements in accordance with the EU directive.

The elimination of food allergens involves the removal of proteins typically present in complex matrices of products, and which include fats, carbohydrates and salts that are often treated with high temperatures. Also, during high-temperature treatment, other non-organic residues can be present on surfaces which come into direct contact with food, and these must be removed from food, but if they are visible during visual control after cleaning, they must be removed.

Proteins are difficult to remove from surfaces, which is why the process of precleaning with cold water and cleaning with warm washing regimes is recommended. Enhanced cleaning regimes to hydrolyse proteins are recommended, using soda with oxidative agents such as the intensifiers, peroxide or chlorine (Table 1).

Special procedures for sanitary processing and methods for maintaining cleanliness of hospitality equipment and inventory must be specified within the program, e.g. removing product residues when work stops (*Popov-Raljic and Blesic*, 2012; 2016). Hospitality facilities must have formal protocols for cleaning and sanitary processing for all rooms (places for preparation, processing and storing foodstuffs), which specify which rooms, appliances or inventory need to be cleaned and how often, in order to reduce the risk of cross-contamination with allergens. Cleaning and sanitation methods vary depending on whether cleaning of equipment and its parts is done in a specified location off the production line (Cleaning-Off-Place — COP), or cleaning takes place immediately and on the production line, with or without disassembling the parts (Cleaning-In-Place — CIP).

The key factors in any effective cleaning regime are chemicals (water hardness, detergent type), contact time, temperature during cleaning and mechanical force used in the process. These key factors are usually shown in the 'sinner's circle' (Figure 1). The sinner's circle shows the actions of one or more individual key factors which can affect each other and balance each other to a certain extent (e.g. less time requires higher temperature and greater concentration of chemicals) (*Basso et al*., 2017).

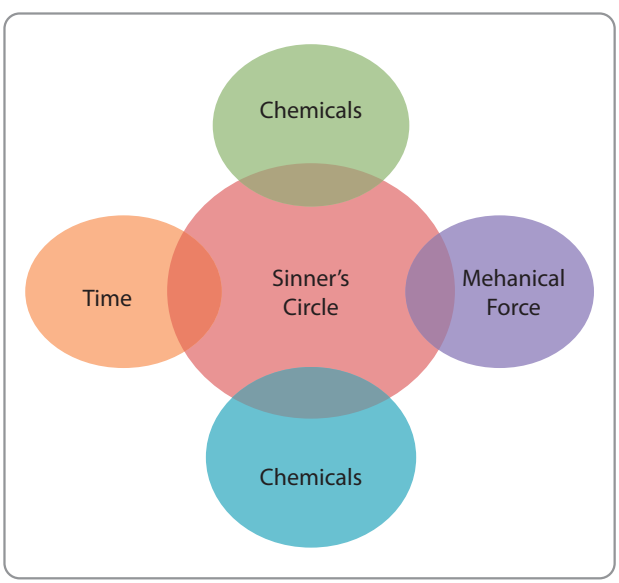


**Figure 1.** The sinner's circle

According to the EU Commission (*Commission Directive*, 2007), materials which come into contact with food must be compatible with foodstuffs and cleaning protocols and must be made of materials that will not corrode after contact with foodstuffs or cleaning agents. The materials must not be toxic or contaminated with microorganisms and they must also be nonabsorbent, wear-resistant, and resistant to breakage, scratching and flaking, which is why work surfaces should not be made of wood or materials such as copper, antimony and other toxic or easily absorbing materials.

During transport of food that can become contaminated from other sources, all cross-carriers must be avoided. If there are not physically separated rooms and transport vehicles for transporting allergen-free foodstuffs and products, as an alternative, they can be transported in closed containers or capped containers.

Cleaning protocols for food allergens must enable the complete removal of allergenic foodstuff residues, e.g. remains of proteins, sulphates and lactose from equipment and inventory in rooms in the hospitality facility where foods are stored, prepared and served (*Flanagan*, 2015).

With the aim of consumer protection as well as defining a protocol for hygienic design, the study was designed to analyse the following: 1) check the labelling of a specified group of products for the presence of chosen allergens, and; 2) establish the allergen status of the work environment after preparation of the meal.

## Materials and Methods

### *Allergen screening*

The Ridascreen, R Biopharm ELISA kit was used to analyse four foods for the presence of egg proteins, β-lactoglobulin, soy and gluten/gliadin. Labels from the four foods (savoury cornbread, pizza pastry, sweet muffin and pork neck) were read to determine whether the foods contained the following declared allergens: egg proteins, milk proteins, gluten or soy.

### *Allergen status of the hospitality kitchen environment*

In order to determine the presence of allergens in a hospitality kitchen, analytical validation was conducted using FLASH® Allergen-Indicator Protein Test swabs (Millipore) on the worktops, knives, meat slicers, convection ovens, worker aprons and worker hands. The hospitality kitchen conducted its everyday business operations and its allergen status was determined after the specified cleaning plan was conducted (including after the validation of correct cleaning had been conducted by employees).

The allergen status of the food preparation environment was determined using FLASH® swabs in work areas after work had finished and after five different wet cleaning protocols:

- Protocol A — a sanitation procedure cold water / warm water / the same wiping cloth after cold and warm water (microfiber) (Table 3);
- Protocol B — a sanitation procedure warm water / warm water with detergent / the same wiping cloth after warm/warm water (microfiber) (Table 4);
- Protocol C — a sanitation procedure warm water / warm water with detergent / changing the cloth after warm water (microfiber) (Table 5);
- Protocol D — a sanitation procedure warm water / warm water with detergent / changing the cloth after warm water (microfiber) / changing the work uniform after food preparation (Table 6);
- Protocol E — a sanitation procedure warm water / warm water with detergent / changing the cloth (microfiber) / changing the work uniform after food preparation / washing hands after food preparation (Table 7).

## Results and Discussion

The presence of allergens in the four food products is shown in Table 2.

In the four examined foods, the detected allergens were declared on the label as required by law. Based on the test results, and similarly to reports by *Jankovic et al*. (2016; 2019), there were no deviations from the declared allergens for this group of products, which is subject to risk because of manipulation of raw materials in the food preparation process and possible cross-contamination and contact.

Used as a part of the HACCP allergen control program, FLASH supports process verification requirements that ensure cleaning methods, which are validated to effectively remove allergens, are consistently applied.

**Table 2.** Presence of declared allergens in examined food products

| TYPE | GLUTEN | SOY | MILK PROTEINS | EGG PROTEINS |
|---|---|---|---|---|
| Savoury cornbread | + | – | – | – |
| Pizza pastry | + | – | – | – |
| Sweet muffin | + | – | – | – |
| Pork neck | – | + | – | – |

The results obtained for allergen detection in the hospitality kitchen after normal daily work and according to the five different cleaning protocols are shown in Tables 3–7.

The results show the best allergen-removal protocols were D and E. After the implementation of protocol D, possible allergen contamination was detected on an employee's apron, which did not occur with protocol E because the employee's uniform was changed. Protocol E, which includes appropriate sanitation procedures, a change of uniform and washing hands after food preparation, could be an excellent allergen mitigation choice, because no food allergens were detected on any of the surfaces examined.

The reduction of the risks presented by food allergens, which everybody in food businesses and consumers face, can be achieved by timely identification of food products which contain materials causing allergic reactions and adequate control in food production and storage process (GMP, HACCP). A hospitality kitchen is a complex and busy system of functionally deployed work areas that undergo wet cleaning, disinfection and sterilization according to protocols. Wet cleaning protocols require high standards of hygienic design for the hospitality equipment, and well-trained staff, which contribute to the removal of allergens and enable safe food production.

In order for the food business to declare that their product does not contain allergens, there must be adequate routine control of the presence of allergens. Such allergen control provides reliable and confirmed information about each specific allergen, and could indicate any possible cross-contamination of the input raw materials (*Grujic*, 2015; *Jankovic*, 2019).

**Table 3.** Allergen detection after cleaning protocol A

| | SANITATION PROCEDURE COLD WATER / CLOTH (MICROFIBER) | SANITATION PROCEDURE WARM WATER / CLOTH (MICROFIBER) |
|---|---|---|
| **Worktop** | +++ | ++++ |
| **Knives** | +++ | +++ |
| **Meat slicer** | +++ | +++ |
| **Convection ovens** | ++++ | +++ |
| **Employee apron** | ++++ | ++++ |
| **Employee hands** | +++ | ++++ |

**Legend:** –, not determined; ++, possible contamination; +++, determined level 1 contamination; ++++, determined level 2 contamination.

**Table 4.** Allergen detection after cleaning protocol B

| | SANITATION PROCEDURE WARM WATER / CLOTH (MICROFIBER) | SANITATION PROCEDURE WARM WATER WITH DETERGENT / CLOTH (MICROFIBER) |
|---|---|---|
| **Worktop** | ++ | ++ |
| **Knives** | ++ | ++ |
| **Meat slicer** | +++ | +++ |
| **Convection ovens** | +++ | ++ |
| **Employee apron** | +++ | +++ |
| **Employee hands** | ++ | +++ |

**Legend:** –, not determined; ++, possible contamination; +++, determined level 1 contamination; ++++, determined level 2 contamination.

**Table 5.** Allergen detection after cleaning protocol C

| | SANITATION PROCEDURE WARM WATER / CLOTH (MICROFIBER) | SANITATION PROCEDURE WARM WATER WITH DETERGENT / CHANGING THE CLOTH (MICROFIBER) |
|---|---|---|
| **Worktop** | ++ | – |
| **Knives** | ++ | ++ |
| **Meat slicer** | +++ | ++ |
| **Convection ovens** | +++ | ++ |
| **Employee apron** | +++ | +++ |
| **Employee hands** | ++ | ++ |

**Legend:** –, not determined; ++, possible contamination; +++, determined level 1 contamination; ++++, determined level 2 contamination.

**Table 6.** Allergen detection after cleaning protocol D

| | SANITATION PROCEDURE WARM WATER / CLOTH (MICROFIBER) | SANITATION PROCEDURE WARM WATER WITH DETERGENT / CHANGING THE CLOTH (MICROFIBER) / CHANGING THE UNIFORM |
|---|---|---|
| **Worktop** | ++ | – |
| **Knives** | ++ | – |
| **Meat slicer** | +++ | – |
| **Convection ovens** | +++ | – |
| **Employee apron** | +++ | ++ |
| **Employee hands** | ++ | – |

**Legend:** –, not determined; ++, possible contamination; +++, determined level 1 contamination; ++++, determined level 2 contamination.

**Table 7.** Allergen detection after cleaning protocol E

| | SANITATION PROCEDURE WARM WATER/ CLOTH (MICROFIBER) | SANITATION PROCEDURE WARM WATER WITH DETERGENT/ CHANGING THE CLOTH (MICROFIBER) / CHANGING THE UNIFORM |
|---|---|---|
| **Worktop** | ++ | - |
| **Knives** | ++ | - |
| **Meat slicer** | +++ | - |
| **Convection ovens** | +++ | - |
| **Employee apron** | +++ | - |
| **Employee hands** | ++ | - |

**Legend:** –, not determined; ++, possible contamination; +++, determined level 1 contamination; ++++, determined level 2 contamination.

## Conclusion

A food allergen risk reduction strategy, which all food businesses and many consumers require, can be achieved by timely identification of food products and raw materials that contain food allergens and adequate control in food production, storage and distribution processes (HACCP). The results of this research show verification of cleaning protocols is required in order to confirm a procedure is efficient. In situations when visual inspections are not practical or sufficient, staff must regularly monitor critical control points. Within the management of food allergens, it is of crucial importance to ascertain the effectiveness of cleaning hospitality equipment, worktops, staff and inventory from food allergens. In order to confirm that an implemented cleaning protocol completely eliminates the allergen

risk and the danger of cross-contamination with food allergens, a complete validation study must be conducted. A documented validation method should be contained in the HACCP plan and be periodically renewed within it in accordance with the dynamics of changes that arise from operations in a hospitality kitchen or from larger changes in business operations (e.g. use of new equipment).

Recommendations for hospitality businesses to protect consumers from allergens are to:

i) develop, improve, implement, maintain and regularly review allergen protocols;

ii) educate employees regarding precisely defined and consistent protocols so they manage allergens properly and produce safe food.

# Kontrola prisustva nutritivnih alergena u ugostiteljstvu

*Milica Aleksić, Jovanka Popov-Raljić, Vesna Đorđević, Mladen Rašeta, Mirjana Lukić, Danka Spirić, Janković Vesna*

*A p s t r a k t: Pružanje usluga u oblast ugostiteljstva je složen postupak sa aspekta sigurnosti zaposlenih i potrošača, zbog čega je sistematski pristup neophodan za uvođenje sistema bezbednosti hrane kroz definisanje kritičnih kontrolnih tačaka (HACCP). Da bi se dobio siguran gastronomski proizvod u pogledu prehrambenih alergena, pored analize sastojaka i / ili gotovog proizvoda, potrebni su validirani protokoli čišćenja u kuhinjama ugostiteljskih objekata kao preduslov uspešne analize rizika i opasnosti. Primena principa redovne kontrole alergena omogućava subjektima u poslovanju hranom da primenjuju odgovarajuće protokole za čišćenje i sanitaciju kako bi umanjili rizik od unakrsne kontaminacije alergenima.*

*Cilj ovog rada je da ukaže na značaj primene potvrđenih režima čišćenja i kontrole sirovina / gotovih proizvoda, kako bi se definisao sistem kontrole na prisustvo nutritivnih alergena, a sve u cilju definisanja dobre higijenske prakse (GHP) i dobre proizvodne prakse (GMP) u ugostiteljskim objektima.*

***Ključne reči:*** *nutritivni alergeni, protokoli čišćenja, kontrola alergena, ugostiteljstvo*

**Disclosure statement:** No potential conflict of interest was reported by the authors.

**Acknowledgment:** This paper was supported by the Innovation Fund of the Republic of Serbia, through the funding of the Project: Allergens as potential contaminants in the food safety system in the hospitality industry, project number 252.

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Paper received: February 13nd2020.
Paper corrected: March 13 nd 2020.
Paper accepted: March 9th 2020.

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**meat technology**
***Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade***

*UDK: 640.4:614.31*
*ID: 16227337*
*https://doi.org/10.18485/meattech.2020.61.1.8*

*Original scientific paper*

# Assuring good food handling practices in hospitality, financial costs and employees' attitudes: A case study from Serbia

*Dušan Borovčanin[1], Nataša Kilibarda[1*]*

*A b s t r a c t: Foodborne diseases are a major threat in the hospitality and foodservice industry. Foodborne incidents registered away from home make up the majority of cases registered in different areas of the world. Since hotels, restaurants and other foodservice establishments are a last line of defence before the food reaches the consumer, the importance of food safety systems in these businesses is vital. Especially in hospitality, successful implementation and efficient application of the HACCP system largely depends on education and motivation of employees who manipulate food. This case study analysed the attitudes of managers and employees in charge of Food & Beverage operations in a corporate luxury hotel in Belgrade. The study showed, on average, the positive attitude of managers and employees towards the issues of food safety and their responsibility in its assurance. Moreover, employees demonstrated a generally high level of satisfaction with their work environment and work conditions and a moderate interest in education and training related to food safety. However, although a HACCP system was documented, the documentation was not systematized and verification of the system was not conducted as planned. Finally, there was no budget defined for food safety related issues such as education and training and external consultations. It is argued that financial costs of food safety, especially those related to education and training, should not be a cause for concern for hotel managers, since evidence from this research suggests they are not significantly impacting the overall food and beverage expenses.*

***Keywords:*** *food safety, good hygienic practice, hotel, cost, financial analysis.*

## Introduction

Food produced in hospitality significantly contributes to the overall food supply. In addition, hospitality is a labour-intensive industry, which contributes significantly to employment, local and international gross domestic product (GDP) and tourism development (*UNWTO*, 2011). Hospitality is understood as a service industry that provides accommodation and food and beverage (F&B) services (*Al Yousuf et al.,* 2015). Foodservice, however, implies activities that produce meals ready for immediate consumption either in conventional restaurants, self-service or take-away restaurants with or without available seating (*United Nations*, 2008). Therefore, the food service industry, defined this way, represents over 60% of establishments serving food under the scope of the food economy, since it includes service in restaurants, bars and cafes, but also restaurants in hospitals, hotels, prisons etc. (*Taylor & Forte,* 2008; *Al Yousuf et al.*, 2015).

The occurrence of foodborne diseases in organisations serving food and beverages is not such a rare occurrence. The reason behind this fact might be due to the heterogeneity of the foodservice industry, which comprises everything from street food handlers to corporate hotels and franchised restaurants that are normally characterised by high internal safety standards (*Jones et al.,* 2008; *Al Yousuf et al.,* 2015). Thus, in the USA for example, for several years now, the highest number of foodborne diseases has been registered in restaurants, i.e. over half of the foodborne diseases (64% to be precise) are registered away from home (*Centers for Disease Control and Prevention (CDC)*, 2019). In the European Union, according to the *European Food Safety Authority and European Centre for Disease Prevention and Control* (*EFSA & ECDC*, 2018), the latest trends show the majority of foodborne diseases are registered within households, followed immediately by those in the foodservice industry.

When it comes to food safety, it is known and confirmed that every link in the food chain holds a certain level of responsibility. Restaurants are generally perceived as the last line of defence before the

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food reaches the consumer (*Kilibarda,* 2019). One of the ways to ensure food safety is through food safety systems like Hazard Analysis and Critical Control Points (HACCP). The HACCP system aims to identify potential hazards and assess where in the production process these hazards could pose a high risk for food safety, but also where and how they can be controlled (or reduced to an acceptable level or eliminated). In Serbia, according to the Food Safety Law RS 17/2019 (*Serbia*, 2019), all subjects involved in food production (in terms of this law, these are hospitality facilities that provide F&B services) are obliged to establish a system for ensuring food safety in accordance with the principles of good hygiene production and practice and HACCP. In this way, both guest and hospitality organisation are assured about food safety and customers can, therefore, enjoy the quality of food and beverages (*Aziz & Dahan,* 2013).

In addition, the application of the HACCP system ensures that food production is carried out as uniformly, economically and efficiently as possible, and with reduced food waste. Furthermore, the employee's awareness and competence level in food handling are also increased with HACCP. In order for the HACCP system to be implemented and enforced efficiently and effectively in a particular food service establishment, it is first necessary that the prerequisite programs of Good Manufacturing Practice (GMP) and Good Hygiene Practice (GHP) are fully introduced and implemented. Both of these systems are recognised internationally by the Codex Alimentarius Commission (*Codex Alimentarius*, 2003).

With the implementation of the above-mentioned programs and compliance with general hygiene principles, adequate procedures are ensured in all food-related activities. However, when it comes to the implementation of the HACCP system in hospitality, problems are often encountered in practice. It should not be forgotten the HACCP system was developed for the needs of the USA space program and was later adopted for use in the food manufacturing industry (*Taylor & Forte,* 2008). The very assumption that the system could be applied without difficulties in hospitality is not rational, since hotels are characterised by several specifics in relation to the rest of the foodservice industry. Some of the specifics are the large amount of input raw materials and final products and the wide variety of recipes, which are caused by the need to adapt to the requirements of guests or due to market competitiveness. All together, this causes an unbalanced volume of food production (that varies daily and seasonally) which is often hard to predict (*Taylor,* 2008; *Taylor & Forte,* 2008). Moreover, another problem is often the physically small manoeuvreing space for the production of several dishes simultaneously (*Eves & Dervisi,* 2005; *Taylor & Forte,* 2008; *Garayoa et al.,* 2011). In addition, a further hospitality-specific issue is the simultaneous production and serving of food (*Garayoa et al.,* 2011; *Taylor & Forte,* 2008).

Small businesses in hospitality often state they experience problems related to financial and infrastructural resources when it comes to HACCP implementation *(Eves & Dervisi, 2005; Taylor & Forte,* 2008). Apart from financial and infrastructural resources, a lack of time and information related to HACCP are also mentioned problem areas (*FDA*, 2006; *Taylor,* 2008). In addition to these obstacles, inadequate space where food production takes place or obsolete equipment that affects performance were also identified as problems (*Wandolo et al.,* 2018). However, in restaurants belonging to large hotel chains, where financial and infrastructural resources should not cause a concern, unskilled staff and the lack of training could be a weak point within the food production process that can lead to increased food safety risk (*Matias et al.,* 2013; *Casolani & Del Signore,* 2016).

Among the most common reasons for the occurrence of foodborne disease in hospitality are raw materials that come from unsafe and unchecked sources, inadequate heat treatment of food, inadequate food storage temperature, improper procedures for defrosting food, contaminated equipment, poor hygiene and/or illness of employees, and inadequate food handling by employees, which is why cross-contamination occurs (*Bolton et al.,* 2004; *Bolton et al.,* 2008; *Adesokan et al.*, 2015; *Jankovic et al.,* 2017).

The human factor is highly significant within cross-contamination in hospitality (*McIntyre et al.,* 2013). The reason for this is because food handlers often have different levels of education, from lower to higher education, but also very different levels of skills and competences. Moreover, hospitality is characterised by a significant degree of seasonality, which often leads to high turnover rates (*Panisello & Quantick,* 2001; *Taylor & Forte,* 2008; *Taylor,* 2008; *Casolani & Del Signore,* 2016), leading further and often to lower levels of training and competencies.

Although, a lack of language skills in the language in which internal training is conducted can be an obstacle to successful understanding of training and adoption of knowledge and practices in the area

of food safety (*Eves & Dervisi*, 2005), good training is still the most widespread strategy that effectively improves food safety *via a vis* the application of good hygiene practice (*Hislop & Shaw,* 2009; *Medeiros et al.*, 2011; *Martins et al.,* 2012; *Rossi et al.,* 2017; *Zanin et al.,* 2017). Successful implementation of HACCP, but also continuous and efficient application of the HACCP system, especially in hospitality, largely depends on education and lasting motivation of employees who manipulate food. In addition, successful and on-going HACCP largely depends on individuals' awareness of the importance of education in this area and the degree of responsibility delegated to them in the process of producing safe food (*Seaman & Eves,* 2006; *Zanin et al.,* 2017).

That is why there is a significant amount of research related to the assessment of knowledge and attitudes about food safety and the application of good hygiene practice by employees in the hospitality industry (*Seaman & Eves,* 2006; *Ko,* 2013; *McIntyre et al.,* 2013; *Adesokan et al.,* 2015; *Rebouças et al.,* 2017; *Barjaktarović-Labović et al.,* 2018; *Al-Kandari et al.,* 2019). Because food handlers have a major role in the prevention of foodborne disease, properly managing human resources is an important step in preparing safe food for consumers (*Eves & Dervisi,* 2005; *Bolton et al.,* 2008; *Rossi et al.,* 2017).

Top management of hotels needs to plan and allocate time and other necessary resources for education and training of employees (*Cates et al.,* 2009; *Kassa et al.,* 2010; *Rebouças et al.,* 2017). It is essential that, while doing so, managers understand the activities performed by every food handler in order to achieve the ultimate goal, which is safe food (*Garayoa et al.,* 2011). Knowledgeable managers can act proactively and with prevention, thereby reducing the potential risks of unsafe food, which are most often caused by inadequate food handling.

However, in order for these activities to be effective and efficient, managers must be qualified, competent and have appropriate knowledge in the field of food safety, which, in practice, is usually not the case. People from a technological, food safety or similar educational and professional background are not normally the majority in hospitality (*Taylor & Forte,* 2008). Even managers themselves do not possess a professional education that would provide them with the appropriate competence and knowledge about jobs related to food safety, which is why they mainly rely on experience (*Ko*, 2013; *Rebouças et al.,* 2017). This is often an obstacle for the successful implementation of food safety policy.

For this reason, the question arises, to what extent, and in what way, do hotels, and especially large hotel chains, really invest in the F&B sector itself, and how much they are really willing to allocate financial investments for internal and/or external staff training related to food safety in the F&B sector, taking into account the specific and overall business and operations of the F&B sector.

## The relative importance of food and beverage in the hotel

The F&B department, alongside the room sector, is among the two most important departments in the hotel business. In many countries, having a full-service restaurant is a mandatory element in the process of hotel categorisation. Nevertheless, F&B in hotels is more often seen by hotel managers as a complementary element. The reason for this attitude lies in the fact that in most cases, the F&B sector does not contribute significantly to the overall hotel revenue relative to the room sector (*Chen & Chang,* 2012; *Yeh et al.,* 2012).

Also, until recently, a large number of guests viewed F&B services in hotels as partially irrelevant, primarily due to the restaurant expensiveness, and, in Serbia, given the large number of alternative, traditional restaurants that can be often found in close proximity to most hotels. In addition, several studies have shown that guests valued F&B relatively poorly when compared to other criteria, such as room cleanliness, location and price, while making their purchasing decision. Therefore, many hotels have shifted their focus towards the room sector, while also shifting the form of food service towards a buffet (which has far lower operating costs) *(Hemmington & King,* 2000), and even, in certain cases, completely outsourcing the F&B division in order to focus on what pays the highest profits, i.e., the room sector (*Hemmington & King,* 2000; *Espino-Rodríguez & Padrón-Robaina,* 2004; *Promsivapallop et al.,* 2012). Almost two decades ago, we witnessed examples of several hotel brands that even abandoned the practice of investing in their own F&B sector, strategically re-locating their facilities and placing new ones near well-known, independent restaurants. This was the case with hotel brands such as Travelodge or Travel Inn (*Hemmington & King,* 2000).

However, recent changes in the global market show signs of significant shifts regarding F&B's importance and position in hospitality. These changes are so big that some hotel chains even see their strategic reorientation precisely in transforming the

F&B sector (*Chesters,* 2017; *Ting,* 2019). These shifts made by large hotel chains may seem unsubstantiated given the foregoing, but it should be borne in mind that the results from the studies above (*Chesters,* 2017; *Ting,* 2019) vary significantly with the size and type of service the hotel provides, as well as the market segment to which guests belong. Also, the fact that guests place such relatively less importance on F&B when booking accommodation is due to the fact that F&B service is something that is often implied (*Albayrak & Caber*, 2015). Their position is to some point confirmed by the evidence which shows the true value of F&B can only be seen in its absence, as evidenced by the results of a study of the operational performance of F&B and non-F&B hotels in New York and California (*Mun et al.,* 2019). The results of this study clearly show the significantly higher performance of full-service hotels than the limited-service hotels.

Finally, in the case of luxury hotels, the quality of F&B and the quality of service have a significant effect on customers' loyalty. Guests who were satisfied with the food and drink at hotels showed a greater degree of readiness to stay at the same hotel, even if they chose other restaurants within the same hotel (*Han & Hyun,* 2017).

## The financial contribution of food & beverage in a hotel: a stumbling point for good handling practices?

Why is F&B profitability so important and how is it related to food safety? First of all, F&B is not the most profitable sector in a hotel. It is characterized by high labour costs, high variable costs and low profitability. The average profitability of the F&B sector ranges between 20–30% in Europe and the Americas, while in the Middle East and Africa it can reach 40% (*Elsen*, 2019a; 2019b; 2019c). This is, however, significantly lower than the profitability of the room sector, which normally ranges between 70–80%, generating more than 60% of total hotel revenue on average (*Elsen,* 2019b, 2019c, 2019a).

On the other hand, ensuring good hygiene practices in a hotel requires significant investment in equipment, technology, staff, and staff education and training, so therefore, one should not be surprised by the manager's restrained attitude when it comes to investing in good hygiene practices. For hotel managers, profitability and cost analysis are of utmost importance in management (*Singh & Schmidgall,* 2002). This is driven by the increasing expectations of investors who expect high returns on their investment (*Maier*, 2016). Is it then possible to invest in good hygiene practices while maintaining or increasing the profitability of the F&B sector?

The importance of safe food and good hygiene practices in hotels is unequivocal in relation to guest safety. However, absolute food safety is unattainable, and therefore, the question arises of optimal investment in safe food and the risk of incidents resulting from inadequate practices. One incident can permanently damage a hotel's reputation and, in the long run, ruin its business reputation and business performance, not to mention the ramifications of the most serious cases of fatal illness and food poisoning that can also carry legal responsibility. For this reason, the implementation of GHP is very important for foodservice, as is the high-quality food that is necessary to earn and keep guest trust and loyalty (*Ko*, 2013).

For managers, one way to invest in food safety is to invest in safety certification. The most common concern before entering the certification process is the cost and impact on the operational efficiency of the department. So far, there are a number of contradictory studies that link certification and its impact on business results (*Aba et al*., 2015; *Al-Refaie et al*., 2012; *Chatzoglou et al*., 2015; *Islam et al*., 2016; *Kusumah & Fabianto*, 2018). A recent study indicates that the introduction of quality management systems (referring to ISO 9001) and hotel certification has nothing to do with business performance, although it has a significant relationship with operating performance (*Duman et al.*, 2019). Methodologically, however, this study did not test whether quality assurance through certification and its impact on operational performance parameters such as guest loyalty and employee satisfaction actually emerges as a mediator in the process of achieving business performance parameters such as total revenue, profitability, occupancy etc. Another study with empirical findings shows that through careful prevention, assessment and reduction of failures in F&B, a hotel can significantly improve its financial performance (*Ramdeen et al.,* 2007).

When it comes to practical implementation, a recent study has indicated that food safety in a hotel depends predominantly on the will of hotel management (Wu, 2012). For this reason, our research focuses on three components: 1) to determine the level of willingness of hotel managers in Belgrade to implement good hygiene practices; 2) to determine the relative level of investment in good hygiene practice, and; 3) to determine the attitude of operational

staff in F&B (which is one of the most critical control points of the whole process) about good hygiene practices.

## Materials and Methods

### *The hotel*

For the purposes of this research, we selected a luxury hotel in Belgrade that is the most developed tourist destination according to its number of arrivals and tourism income (*Ministry of Trade, Tourism and Telecommunications*, 2020). The hotel operates as a part of one of the world's leading corporate chains and uses the franchise of the premium brand of that hotel chain. The hotel has a staff of 105 employees, 36 (34%) of which are employed in the F&B sector. In addition to the kitchen, F&B runs two hotel restaurants, a lobby bar and a multifunctional banquet hall that can be divided into several units where food and drink can be served for various types of events.

The kitchen is a unique functional unit consisting of several work areas in which the technical-technological process of food preparation takes place. It fulfils all technical, technological, sanitary-hygienic and organizational-staff requirements in accordance with the current regulation (*Serbia*, 2016). The hotel has an established and documented HACCP system. However, the documentation is not systematized and the verification of the system is not conducted systematically and with plan. Therefore, this hotel is not HACCP certified. Also, the hotel does not have certification for either the international standard for quality management system (*SRPS ISO*, 2015) or food safety standard (*SRPS EN ISO*, 2018).

### *Research sample and method*

A qualitative approach was applied in this research since it is a case study, and it is one of the first studies of this type performed in Serbia. After a site inspection, interviews were conducted with the manager of the F&B sector and the kitchen head chef to determine the will and attitudes of the top executives in relation to good hygiene practices.

In order to conduct quantitative research and collect the data, a questionnaire of 10 questions with a five-point Likert-scale was created. Respondents had to answer by rounding off one of the offered choices. All respondents participated in the research willingly, and they were informed in advance about the research and that the data would be used exclusively anonymously and that they could withdraw from the research at any time without any consequences. The research was conducted in accordance with the ethical principles and code of conduct prescribed by the American Psychological Association (*APA*, 2017).

The questionnaire consisted of two parts. The first part consisted of questions related to the socio-demographic status of the respondents (gender, age, work experience and education).

The second part of the questionnaire consisted of 10 questions related to employees' attitudes. The questions were related to: 1) the attitude of employees on their role in the process of delivering safe food, then; 2) the number and quality of on-the-job training sessions in relation to good hygiene practice and food safety, and; 3) the satisfaction of employees with their work conditions, personal income and the equipment they use.

Finally, by rounding off one of the answers to the last question, the respondents had to decide on what was, according to their opinion, a major difficulty when it comes to the adequate implementation of good hygiene practice. Subsequently, questionnaires were distributed to all employees in the F&B department, of which 29.4% were returned completely filled.

After data collection, in order to perform further analyses, the data were coded and transformed into a numerical matrix. Data from the first part of the survey were transformed into nominal and ordinal scales according to respondents' answers and their frequency. The Likert scale answers and results were analysed as follows: from 1 to 1.5 — very negative attitude, from 1.5 to 2, 5 — moderately negative, from 2.5 to 3.5 — neutral attitude, from 3.5 to 4.5 — moderately positive, from 4.5 to 5 — very positive attitude about food safety on trips. The collected answers to the last question were grouped and expressed as a percentage frequency.

## Results and Discussion

In an interview with the hotel's F&B manager, he demonstrated a positive attitude towards food safety. His attitude could be clearly evidenced by his statement *"I am aware of the importance of safe food and good hygiene practices and the role of my team in ensuring it. For this reason, several times my team and I have launched the HACCP certification initiative. Although the hotel is not certified yet, we strive to deliver quality the same way as if the hotel was certified."*

This attitude from the F&B manager is very encouraging and important. The literature points out that a positive attitude of managers towards the implementation and enforcement of good hygiene practice can have a significant impact on its adequate application by the employees, because it has a positive effect on the transfer of knowledge, feedback of information, self-control and individual commitment to the implementation of GHP (*Eves & Dervisi,* 2005; *Seaman & Eves,* 2006; *Ko,* 2013*; Casolani & Del Signore*, 2016). Employees' motivation and approval from management are also crucial for successful food safety management. Without any demonstrated commitment from management, the fact is that investing in employee training alone would not lead to improved practices, and thus, to increased food safety (*Seaman & Eves,* 2006).

As the biggest obstacle to certification, the F&B manager cited the financial costs, while in addition to costs, the head chef also cited the excessive number of lists and documentation that operationally slows the team down. The chef indicates the additional imposition of administrative duties on the members of his team, stating that *"when a hotel is running with full capacities and when there is a big event staging in the hotel apart from regular guests, work in the kitchen becomes extremely demanding. Introducing additional tasks that certification might bring would further slow us down, and the speed of meal production is very important in these situations."*

This attitude is not surprising (indeed, it is expected) given the specificity of food production in hospitality where a large number of different dishes is produced. The process of food production and food serving is simultaneous, and there are often changes within the menu, which are an aggravating circumstance when it comes to application and certification of the HACCP system (*Ko*, 2013). Similar attitudes were discovered in qualitative research done by Eves & Dervisi with managers from the foodservice industry (*Eves & Dervisi,* 2005). Namely, HACCP itself cannot make food safe, but its proper implementation can. In order to implement the system properly, it is necessary to remove barriers, such as for example, the perception of management and employees that HACCP means both additional and unnecessary administration that can only slow down the process.

Although young (staff were 28 years old, on average), the F&B team had several experienced individuals who train younger staff, especially during the socialization process. Both managers demonstrated a positive attitude towards safe food training. They stated that additional training for their team would be beneficial. The main obstacle is the lack of a predefined budget for these purposes and the definite and mandatory number of training sessions that must be completed. The manager of the F&B sector further states: *"The corporation insists on other types of training such as upselling and cross-selling training, but certainly more training about food safety would be much more useful for us."*

For the successful implementation and maintenance of the HACCP system and GHP, it is of great importance that there are predetermined financial resources are allocated for costs related to the monitoring and record-keeping, staff training, costs of corrective actions when the critical limit is exceeded and costs of consultations that may be required. Often, the need to increase sales in the F&B sector and generate higher revenues can push aside training and improving knowledge to refresh good hygienic practice (*Panisello & Quantick,* 2001). In the worst-case scenario, this can cost much more, as lack of training can be a significant risk in hospitality when it comes to the occurrence of foodborne disease, as mentioned above.

Since this is a new and luxurious hotel, care had been taken to meet all infrastructure requirements and to reduce the risk of cross-contamination to a minimum, which is one of the main prerequisites for adequate and proper implementation of GHP (*da Cunha et al.,* 2013; *Zanin et al.,* 2017). As it is a high-end luxury hotel, the equipment in the hotel is at a satisfactory level, while investment in the equipment, in addition to the on-going maintenance, is made every two years. The hotel possesses a licence for software aimed to control the cost of F&B materials, i.e. *Materials Control*, one of the world's leading *Oracle* solutions for this purpose. However, there is no software system for temperature monitoring that provides cold chain information, although there are thermometers on every device in the kitchen that are manually monitored and recorded. Also, the hotel does not have any software that monitors inventory expiry dates. However, investing in a system that would automatically and continuously track the temperatures of refrigeration devices could significantly save time for employees, especially since 50% of the respondents, when asked what they consider to be the biggest obstacle to doing business under the GHP, stated too many responsibilities per employee (Figure 1). On the other hand, software that monitors products' expiry

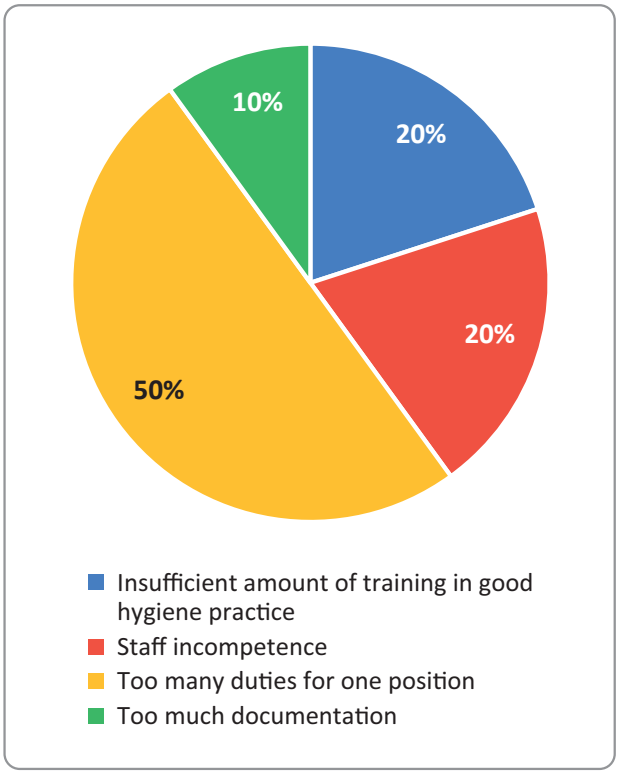


**Figure 1.** Major difficulties reported by employees regarding adequate implementation of good hygiene practice

dates could significantly save employees' time in terms of inventory control necessary to the first-in first-out principle. This could positively affect total costs in F&B, with less waste of expired foods/products. This would enhance the food safety management and management of food waste (*Kilibarda et al*., 2019; *Kilibarda,* 2019*)*.

The demographic structure of the respondents was fairly balanced in relation to gender. Female respondents made up 60% of our survey respondents, while male respondents comprised 40% of the sample. The average age of respondents in the F&B department was 28.2 years, meaning this is a relatively young team. The average number of years of experience in the F&B business from our respondents was 6.4 years, which was clearly related to the age structure of the respondents. When it comes to educational level, this was balanced between respondents having attended high school and having a bachelor's degree (60:40 in favour of high school), which is in line with the usual education level in hospitality in Serbia (Table 1).

To ensure food safety in the F&B sector in hotels, it is fundamental to establish and maintain good hygienic practice (*Martins et al.,* 2012; *Serafim et al.,* 2018). Training of food handling employees is the most widespread strategy that effectively improves food safety (*Hislop & Shaw,* 2009; *Medeiros et al.,* 2011; *Martins et al.,* 2012; *Soares et al.,* 2012; *da Cunha et al.,* 2013; *Zanin et al.,* 2017), given that the majority of foodborne diseases in hospitality occur as a result of inadequate food handling (*Zanin et al.,* 2017; *Barjaktarović-Labović et al.,* 2018). However, the training itself can lead to improvements in the application of good hygiene practice only if the transferred knowledge leads to changes in practice (daily activities) or attitudes of employees in the workplace *(Seaman & Eves, 2006; Zanin et al.,* 2017). Two studies have shown no positive correlation between employees' knowledge and their daily practice (*Soares et al.,* 2012; *da Cunha et al.,* 2013). The reason for this may be that the proper application of adequate practice in routine work depends on the motivation of employees, their awareness of their job importance and the level of responsibility they have in the process of food production, regardless of their level of knowledge *(Seaman & Eves, 2006*; *Zanin et al.,* 2017).

Our research aimed to determine the attitudes of employees in relation to responsibilities and training related to food safety. Attitude is a crucial factor influencing employee behaviour and practice, since a positive attitude can reduce the incidence of foodborne disease. Attitude represents a link between knowledge and practice (*Ko,* 2013; *Manning,* 2018), i.e., employees who have more knowledge are more likely to apply it in practice if they have a positive attitude and *vice versa* (*Zanin et al.,* 2017; *Al-Kandari et al.,* 2019), the training of employees, and their gaining a higher level of competence, will not lead to improved practice if the attitude of employees towards training is negative (*Bolton et al*., 2008).

In our research, the answers to the first three questions, which were related to the employee's attitude towards responsibility for food safety in the

**Table 1.** Employees' characteristics (% of respondents)

| Gender | | Age | | Education | | Work experience | |
|---|---|---|---|---|---|---|---|
| Female | 70 | < 25 years | 50 | High school education | 60 | < 10 years | 70 |
| Male | 30 | > 26 years | 50 | Higher education | 40 | > 10 years | 30 |

**Table 2.** Questionnaire administered to ascertain employees' attitudes towards food safety and the average score attained

| Questions | Average score |
|---|---|
| 1. Food handling employees have a significant level of responsibility in terms of food safety | 4.6 |
| 2. Education and training of employees related to good hygiene practice are important activities of employees in the F&B sector | 4.7 |
| 3. Education and training I had related to GHP impacted significantly and enabled me to understand the importance of GHP in food safety assurance | 4.6 |
| 4. Education and training I had on GHP impacted significantly my personal sense of responsibility on workplace | 4.1 |
| 5. I consider that additional education and training on GHP would change my behaviour and daily routine | 3.8 |
| 6. I consider that additional education and training are necessary for F&B employees | 4.0 |
| 7. I am satisfied with the number of education and training sessions on GHP provided by my employer | 4.1 |
| 8. I am satisfied with the quality of education and training on GHP received so far from my employer | 4.0 |
| 9. I am satisfied with my personal income | 4.4 |
| 10. I am satisfied with my work uniform and protective equipment (quantity and quality) provided by my employer | 3.5 |

workplace and towards the training they received on the application of good hygiene practice, demonstrated high average scores (Q1 = 4.6, Q2 = 4.7, Q3 = 4.6) (Table 2). It can be argued that the F&B staff are aware of their responsibilities in the workplace when it comes to food safety and that they have a very positive attitude towards the training they had regarding the application of good hygiene practice. *Ko* found similar results in his surveys of the attitudes of food handlers in restaurants (2013). This attitude of the respondents is very satisfactory and encouraging given the recommendations by the *Codex Alimentarius* (2003). The attitudes of employees who do not consider training important and useful are considered especially risky for food safety (*Zanin et al.,* 2017).

The average score from the respondents about the degree to which training enabled them to consider the degree and importance of personal responsibility in the workplace when it comes to food safety is fairly positive (Q4=4.1) (Table 2).

The reason for the fact that a distinctly positive attitude has not been established as in the previous three questions could be that the choice of formal education in the hospitality profession already affects the development and perception of the importance of this type of responsibility in the workplace. It is also interesting that moderately positive attitudes were established regarding the statement that attending more training on GHP would improve behaviour in the workplace when it comes to its implementation (Q5 = 3.8) and to the statement that employees need more training on applying GHP (Q6 = 4.0) (Table 2).

Although employees evaluate and consider their responsibility in the workplace and the training they have attended very positively, they expressed only a moderately positive attitude towards attending a larger number of training sessions as well as the usefulness of those sessions. This attitude could be improved primarily through detailed planning and design of training, because the success of training largely depends on the method of implementation. Employees should be provided with training containing less theory and more practice, which often results in higher effectiveness (*Medeiros et al.,* 2011; *Zanin et al.,* 2017).

When planning training for employees, it is necessary to take care that training sessions do not last for too long, as this can create resistance among employees. Training should be, rather, practical, short-lasting, periodic and relevant (*Soares et al.,* 2012; *Adesokan et al.,* 2015).

Analysing the responses of the F&B employees, a moderately positive attitude towards the number and quality of training sessions on the application

of GHP provided by the hotel for employees was determined (Q7=4.1, Q8=4.0) (Table 2). In the hotel selected for this case study, based on information received from the manager, training on the application of GHP is conducted only for newcomers (recently hired staff). However, for employees in the F&B sector, continuous and/or periodic training on the application of good hygiene practice or periodic refresher training courses are not organised (*Codex Alimentarius*, 2003). These types of training are of essential importance from the aspect of updating knowledge and maintaining motivation levels (*Casolani & Del Signore,* 2016).

Despite the moderately positive average ratings of employee satisfaction when it came to the quality and number of training sessions, it can be concluded that in fact, these moderately high average ratings are cause for potential concern. Why?

The fact is that each F&B staff member at the hotel has had only one training session on the application of GHP since they were hired. It was previously pointed out that trained staff helps guarantee food safety, that training is an urgent need, and that the frequency of errors during work increases with the lack of training (*Clayton & Griffith,* 2004). Furthermore, the knowledge, once acquired, declines over time (*McIntyre et al.,* 2013). According to *Soares et al.* (2012), training is an integral part of creating a positive culture of food safety, and must be conducted periodically to reduce the risk of foodborne disease. Our results suggest it is necessary to conduct further research to determine the level of employees' knowledge about food safety and the adequacy of their applying GHP during routine work.

In general, it could be argued that employee satisfaction with work conditions and personal income was positive (Q9 = 4.4) (Table 2). A moderately positive attitude of employees was also determined when it came to employee satisfaction with work uniforms and equipment (Q10 = 3.8) (Table 2). *Clayton & Griffith* (2004) point out that the attitudes of F&B employees are in fact determined and positively correlated with resources and infrastructure of the hotel. Also, employee satisfaction, both in terms of earnings and work conditions, affects the consistent application of GHP, and thus, increased satisfaction reduces the risk of unsafe food (*Rebouças et al.,* 2017; *Zanin et al.,* 2017).

The hotel used for this research is in the top 10 hotels in Serbia measured by the level of sales, according to the Business Registers Agency of Serbia. The profitability of the F&B sector in the hotel is at the level of 30%, which is in line with the European average and can be considered as a positive indicator of the business approach.

Nonetheless, results from Table 3 show the hotel used for this case study had a financial struggle to achieve an operating profit during the last five years. However, its F&B sector performs well, as it achieves a stable operating profit. It is reasonable to understand that adding additional costs to the operation in a situation when hotels struggle to achieve and maintain profit might be inconceivable for hotel managers. Nevertheless, additional costs of education and training

**Table 3.** Financial results of the hotel and cost of education and training in food safety

| | 2014 | 2015 | 2016 | 2017 | 2018 | Sum of last 5 years | 5 year average |
|---|---|---|---|---|---|---|---|
| Total revenue (€)* | 99,696 | 2,528,388 | 4,559,476 | 5,997,593 | 6,016,823 | 19,201,976 | 3,840,395 |
| Total expenses (€)* | 2,232,984 | 4,932,716 | 5,460,626 | 6,936,307 | 5,685,192 | 25,247,825 | 5,049,565 |
| F&B revenue (€)** | 744,328 | 1,644,239 | 1,820,209 | 2,312,102 | 1,895,064 | 8,415,942 | 1,683,188 |
| F&B expanses (€)** | 521,030 | 1,150,967 | 1,274,146 | 1,618,472 | 1,326,545 | 5,891,159 | 1,178,232 |
| F&B operating profit (€)** | 223,298 | 493,272 | 546,063 | 693,631 | 568,519 | 2,524,783 | 504,957 |
| Total cost of education and training related to food safety (€)** | 2,250 | 2,250 | 2,250 | 2,250 | 2,250 | 11,250 | 2,250 |
| Cost of education and training in total expanses (%) | 0.101 | 0.046 | 0.041 | 0.032 | 0.040 | | 0.052 |
| Cost of education and training in total revenue (%) | 2.257 | 0.089 | 0.049 | 0.038 | 0.037 | | 0.494 |
| Cost of education and training in F&B expenses (%) | 0.432 | 0.195 | 0.177 | 0.139 | 0.170 | | 0.223 |
| Ratio of cost of education and training to F&B profit | 1.008 | 0.456 | 0.412 | 0.324 | 0.396 | | 0.519 |

**Legend:** *According to the Business Registers Agency of Serbia; **According to data obtained

related to food safety would increase the F&B total expenses by less than 0.5% and would additionally assure the safety and quality of the service provided (Table 3).

On the other hand, excellent work conditions prevail at this hotel. This is confirmed by the average employee rating, which averaged 4.4 on a scale of 1–5 (Table 2). The average gross monthly earnings in the F&B sector at this hotel are net 75,546.75 RSD, which is higher than the national average and the average in the hospitality industry (*Statistical Office of the Republic of Serbia*, 2019).

We see this as an example of good practice, because hospitality is known to be distinguished by poor work conditions for employees (*Baum,* 2015). For this reason, we see investment in employees, their income, their on-the-job training and the good work conditions as a way to ensure greater hotel profitability, as shown by the results of previous studies (*Yee et al.*, 2008; *Chi & Gursoy,* 2009). This is in line with what previous studies have already shown, i.e., satisfied employees in a hotel feel enthusiastic and inspired by their job, so tend to provide better service quality to customers (*Kong et al.,* 2010), and content employees also tend to be more intent on staying in the company (*Chiang et al.,* 2008). This is extremely important in hospitality, as it records high employee turnover rates across the industry. There are estimates that the average turnover of frontline employees in a year in hospitality fluctuates around 65% (*Myers*, 2005). Low incomes and high employee turnover rates are negatively correlated with the efficiency of training and adequate adoption of practices, which can negatively affect food safety (*Seaman & Eves,* 2006; *Garayoa et al.,* 2011; *Casolani & Del Signore,* 2016; *Zanin et al.*, 2017). In this study, this was not the case, and the high satisfaction level of employees was largely related to their personal incomes, followed by the excellent work conditions.

## Conclusion

Lack of employees' motivation is often identified as one of the barriers to successful GHP and HACCP implementation. In this case study, a high level of employees' satisfaction was identified, which was explained by several factors, primarily personal income, and the work environment and conditions. It is clear that, in addition to the positive attitude from the management, this is also reflected in the positive attitude of the employees and their evaluations of their own responsibilities for food safety. However, the facts that the documentation of the HACCP system is not systematized and the verification of the system is not planned clearly indicate the lack of training, primarily of the management of the F&B sector.

Additionally, worrying are the lack of available, dedicated training funds in line with the lack of training for all F&B employees. Apart from the initial financial investments (infrastructure), and current investments dedicated to the costs of equipment maintenance and monitoring of equipment performance and personal income, there are no other investments. These would refer to consulting services and external training services, which are obviously necessary for more successful F&B operations in terms of food safety

This should be underlined, since evidence from our research suggest that these costs (costs for external training and consulting services related to food safety) would amount to less than 0.5 % of the F&B sector's total costs, on average, for the last 5 years of operation. Bearing in mind the importance and impact that food safety has on hospitality, the real financial costs of proper HACCP implementation and food safety excellence should not be a cause for concern.

The limitations of this research are related to the fact this is a case study, i.e., the research was conducted in only one hotel. Therefore, it is necessary to determine the attitudes in a broader sample of employees from the hospitality industry. Further research should determine the degree of employees' food safety knowledge and the degree to which they implement their knowledge in daily practice. Finally, employees' knowledge, attitudes and implementation should be studied in relation to factors such as job satisfaction, work environment and personal income. It is of great importance to further expand similar research to other business operating in the hospitality industry, especially small businesses, where very few financial resources are normally allocated for food safety.

# Obezbeđenje dobre higijenske prakse u ugostiteljstvu, finansijski troškovi i stavovi zaposlenih. Studija slučaja iz Srbije

*Dušan Borovčanin, Nataša Kilibarda*

*A p s t r a k t: Bolesti prenosive hranom predstavljaju značajan razlog za zabrinutost u sektoru ugostiteljstva, kao i u svim subjektima koji obavljaju delatnost usluge hrane i pića. Razlog tome je što se poslednjih godina najveći broj bolesti prenosivih hranom, registruje prilikom konzumacije hrane van kuće, u različitim delovima sveta. Kako hoteli, restorani i drugi subjekti koji posluju hranom predstavljaju poslednju liniju odbrane hrane pre nego što ona dođe do potrošača, sistemi bezbednosti hrane implementirani u svim ovim subjektima su od velike važnosti za osiguranje bezbednosti hrane. Poznato je da naročito u ugostiteljstvu, uspešna implementacija kao i efikasna primena HACCP sistema, najviše zavisi od stepena obučenosti i motivisanosti osoblja koje rukuje hranom. Ova studija slučaja imala je za cilj da analizira stavove menadžera i zaposlenih u sektoru hrane i pića u jednom luksuznom, korporacijskom hotelu u Beogradu. Rezultati studije pokazali su da menadžeri i zaposleni u proseku imaju pozitivan stav prema bezbednosti hrane i odgovornostima koje imaju kada je reč o bezbednosti hrane na radnom mestu. Takođe, zaposleni su pokazali visok stepen zadovoljstva uslovima rada i okruženja, ali i umereno izraženo interesovanje za obuke i treninge koji se tiču bezbednosti hrane. Međutim, u ovom hotelu HACCP sistem je dokumentovan, ali dokumentacija nije sistematizovana, a provera efikasnosti sistema se ne izvodi planski. Dodatno, utvrđeno je i da ne postoji jasno opredeljen budžet za aktivnosti koje se odnose na osiguranje bezbednosti hrane, kao što su na primer, obuke i treninzi osoblja, kao i eksterne konsultantske usluge. Zaključeno je da finansijski troškovi koji bi se odnosili na osiguranje bezbednosti hrane, posebno oni koji se odnose na edukacije i treninge zaposlenih, ne bi trebalo da predstavljaju dodatno opterećenje za menadžere, budući da rezultati* istraživanja ukazuju na to da oni ne utiču značajno na ukupne troškove sektora hrane i pića hotela.

***Ključne reči:*** *bezbednost hrane, dobra higijenska praksa, hotel, troškovi, finansijska analiza.*

**Disclosure statement:** No potential conflict of interest was reported by the authors.

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Paper received: May 22th 2020.
Paper accepted: June 9th 2020.

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**MEAT technology** : scientific journal / editor in chief Vesna Z. Djordjevic. - Vol. 57, No. 1 (2016) - . - Belgrade : Institute of Meat Hygiene and Technology, 2016- (Beograd : Naučna KMD). - 30 cm

Dva puta godišnje. - Je nastavak:
Tehnologija mesa = ISSN 0494-9846
ISSN 2466-4812 = Meat technology (Belgrade)
COBISS.SR-ID 225196812