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Review paper

Does Serbia need mobile slaughterhouses?

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ABSTRACT

Mobile slaughterhouses were first mentioned in 1960 and were used to process the carcasses of hunted deer. Today, the use of these facilities for slaughtering animals, processing carcasses and cooling meat is primarily for the slaughter of farm animals (dairy cows) that have difficulty moving and cannot tolerate long transport durations. These mobile units are also used nowadays for the slaughter of lambs (prior to weaning) that are not used to drinking water, and when the transport is longer than 10 hours. Most often, the use of mobile slaughterhouses is related to the well-being of animals, i.e., to mitigating the numerous stressful situations that animals go through from their place of residence to the stunning box. However, meat quality is also a factor in the use of these slaughterhouses. Consumers who are particularly interested in animal welfare are ready to pay a higher price for meat obtained from animals that are exposed to less stress (which typically arises from long transport, lack of food and water, overcrowded vehicles or weather conditions). The other main advantage of mobile slaughterhouses is, in addition to reducing the length of transport, the forging of direct connections between breeders and slaughterhouses (no intermediaries, buyers). This is of particular importance for the mountainous areas of Serbia, where small ruminants are mostly raised in peasant households with a small number of animals. The use of mobile slaughterhouses would reduce the number of animals slaughtered by households (small ruminants, piglets) outside veterinary supervision. For the application of mobile slaughterhouses, a good knowledge of the raw material base (species and number of animals, volume of animal feed production), demographic data, roads, energy, water resources, etc., is necessary. Mobile slaughterhouses must meet all the operating conditions that apply to stationary slaughterhouses.

1. Introduction

Agriculture and food production have always been of particular importance to Serbia, not only for the food security of its own population, but also for the export of surplus food. Soil and climatic conditions provide Serbia with good opportunities for the production of foods of plant and animal origin. The main foods of animal origin in Serbia were pig meat for many years, then beef, and from about fifty years ago, poultry meat. Today, poultry meat accounts for 40% of total meat production worldwide. Although the percentage of small ruminant meat production among the total meat production in Serbia is small, it is not insignificant. It is particularly important for the hilly and mountainous regions of Serbia, rich in grassy areas that are best utilised by sheep and goats. Given the breed composition (and different strains of the breeds), sheep are typically raised in hilly and mountainous areas for meat, milk and wool. To increase the volume of small ruminant breeding in Serbia, and thus increase food (meat, milk) production, it is necessary to analyse the raw material base (production

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of animal feed, demographic data, number of sheep and goats). In addition, to obtain meat, especially in the case of increased animal breeding, it is necessary to build specialised slaughterhouses, which could be standard or mobile, and which would be close to the raw material base. These facilities and the production of meat in them would be under constant veterinary control, which would ensure the safety of meat and reduce the occurrence of uncontrolled slaughter of animals and meat thereof entering the meat trade.

2. Historical overview of animal slaughter

Until about 13,000 years ago, Homo sapiens was a hunter (and fisher) and gatherer. Domestication of animals and cultivation of the land has been applied gradually and non-uniformly in all parts of the world since that time. Even today, about 60 tribes continue to live in the same way they lived in the Neolithic. The transition from the hunter/gatherer way of life to settled agriculture was, therefore, slow and long-lasting. In fact, agricultural production became the basic occupation of man and became widespread a mere 7,000 years ago. That is a much shorter time than the time for which man was a hunter/gatherer (~200,000 years) and when people lived without being tied to one place, but moved and settled where food sources were richer. The first civilisations (organised states) arose in the area of Mesopotamia, between two rivers, the Euphrates and the Tigris, in the area of today's Iraq, Kuwait, Turkey and Syria. Ancient civilisations include Egyptian, Greek, Roman, Mayan, and the Indus Valley (Baltić et al. 2010). All contained settlements and cities with a large number of inhabitants. Thus, Uruk (Sumer, later Babylon) between 4,000 and 3,000 BCE had 80,000 inhabitants. It spread over an area of six square kilometres and was surrounded by a wall. Those first civilisations were states that had regulations (e.g., Hammurabi's laws), and part of those regulations related to the slaughter of animals (Baltić and Marković, 2017). For later Christian civilisations, those regulations were contained in the Holy Scriptures of the Old and New Testaments. In the third book of Moses (Chapter 22) it is written, "let him offer a willing male of cattle, or of a sheep, or of a goat", "do not offer anything on which there would be manna, because it would not be acceptable to you". Further in the book, more detail is presented about faults, about the prohibition of castration, about the time that a lamb and calf should spend with the mother, about the prohibition of slaughtering cows, sheep and goats on the same day as their calves, lambs and kids, and that the meat should be eaten on the same day and not left for tomorrow (Daničić and Karadžić- Stefanović, 1973.) Other holy books (Koran, Talmud) also talk about slaughtering cattle. In Ancient Greece and the Roman Empire, animals were slaughtered in designated places, usually under the control of a priest. In Rome at the end of the 4th century, state officials controlled livestock markets, slaughterhouses and the meat trade. In the Middle Ages, there were regulations on animal slaughter and slaughterhouses. In 1321, in Kotor (today's Montenegro), a regulation was passed according to which animals had to be slaughtered in slaughterhouses. Even in the Serbia of the Middle Ages, there were regulations on animal husbandry and the meat trade (Dušan's Code, Law on Mines; Vuković, 1992; Baltić and Đorđević, 2019).

3. The road to modern slaughterhouses

At the end of the 18th and especially in the 19th century, regulations were passed on animal slaughter and slaughterhouses in France and Germany. Thus, it was stipulated that every town in France must have a communal slaughterhouse. The need for the construction of slaughterhouses arose from the fact that by 1804, the world had a billion inhabitants and that increasing numbers of people lived and worked in cities, i.e., industrial centres that needed to be supplied with food, including meat. The meat and meat products supplied had to be safe for human consumption and in sufficient quantity. Without large-capacity slaughterhouses, it was impossible to supply large cities with meat. However, the refrigeration systems were insufficient to store the meat. In practice, the meat had to be circulated to consumers within 12 to 24 hours, especially in the warmer season (*Vuković*, 1992; *Baltić et al*. 2010).

At the beginning of the 19th century, among the ten largest cities in the world, three were in Europe (London, 900,000 inhabitants; Paris, 547,000; Naples, 430,000) and the other seven were in Asia (from Osaka, 300,000, to Beijing, 1,100,000). Moscow had 238,000 inhabitants, Vienna 231,000 and Berlin 172,000. In Western Europe, from 1820 to 1825, 12% of the population lived in cities with more than 10,000 inhabitants, and in 1890, that percentage had risen to 31% (*Osterhamel*, 2022). Belgrade had about 30,000 inhabitants in 1777, but ~4,000 in 1834, which was a consequence of wars with the Ottoman Empire; ten years later, 10,000

Serbs, 5,000 Turks, 1,383 Jews and 900 foreigners (mostly Germans) lived there. In Serbia at that time, in addition to Belgrade, the largest cities were Požarevac (3,733 inhabitants), Jagodina (3,166), Šabac (2,936) and Kragujevac (2,316) (*Gavrilović*, 1846). In 1739, there were 30 slaughterhouses in Belgrade, individually owned by Serbs, Germans, Jews, Turks and Armenians. At that time, meat products produced by German-owned slaughterhouses appeared on the market. There is no information on regulations related to animal slaughter, slaughterhouses or the meat trade. Regulations on animal slaughter, slaughterhouses and the meat trade appeared during the time of Karađorđe (1804–1813), and especially during the two reigns of Prince Miloš Obrenović (until 1860). A new, modern slaughterhouse was built in Belgrade in 1855, and new rules on cattle slaughter were adopted in 1888. The Serbian Joint Stock Company for Cattle Slaughter was founded in 1897, became an exporter of meat from Serbia, and thus, 1,800 tons of meat products (ham, bacon) were exported to Great Britain, France, Switzerland, Italy, Algeria and, until the Customs War, to Austria-Hungary (*Labudović et al.,* 1979). In 1914 Serbia exported 9,076 tons of meat products, and right before the beginning of World War I, thanks to the help of Mihajlo Pupin, it exported 5,000 tons of pork fat to the United States. Between the two world wars, the Kingdom of Serbs, Croats and Slovenes did not have enough slaughterhouse capacity to export large quantities of meat. The Kingdom of Serbs, Croats and Slovenes was unevenly developed at this time, as evidenced by the fact that before World War II, the largest number of slaughterhouses was in Slovenia (306), with the other entities having smaller numbers: Croatia (147), Serbia proper (70), Vojvodina (73), Bosnia and Herzegovina (76), Kosovo and Metohija (10), Macedonia (27) and only a few in Montenegro. Of the slaughterhouses in Serbia, 10 were export slaughterhouses. In Serbia, slaughterhouses were built in the late 19th and early 20th centuries in Jagodina, Velika Plana, Mladenovac, Belgrade, and after World War I in Šid, Banatski Karlovac, Čoka, Novi Sad, Subotica, Kraljevo and Kruševac. In 1948, there were 153 slaughterhouses in Serbia, and in the late 1980s, there were about 550, of which 21 were export slaughterhouses. After World War II, more modern slaughterhouses were built in Yugoslavia, and in Serbia alone there were 20 industrial facilities for slaughtering cattle and processing meat registered for export (*Baltić and Đorđević*, 2019).

4. Serbia today — animal slaughter

From 1990 and in the following 35 years, most of the export slaughterhouse facilities in Serbia stopped working. Today, Serbia is an importer of meat for processing and an importer of live animals. There are many reasons for this state of affairs (the disintegration of Yugoslavia, wars, sanctions, reductions in the numbers of farmed animals, especially pigs and cattle, aging of village populations). In Serbia today, there are about 300 registered slaughterhouses with different scopes of production for animal slaughter and meat processing, so they are accordingly divided into craft and industrial establishments.

The numbers of livestock species in Serbia have been in constant decline since 1990. In 2021–2023, cattle average annual numbered 795,000, pigs 2,559,000, sheep 1,771,000, goats 178,000 and poultry 14,814,000. These numbers are each far smaller number than in the previous 50 years. In that period (from 2021 to 2023), the annual average number of cattle slaughtered in all categories was 316,000, pigs 5,202,000, sheep 1,855,000 and poultry 66,422,000. An annual average of 168,000 cattle, 1,190,000 pigs, 217,000 sheep and 64,120,000 poultry were slaughtered in slaughterhouses. From the above data, it can be seen that 53% of cattle, 23% of pigs, 12% of sheep and 96% of poultry were slaughtered in slaughterhouses. Although the slaughter of cattle outside slaughterhouses is prohibited, almost half the cattle in the country are not slaughtered in slaughterhouses, which can be explained by the fact that the vast majority of calves, especially those of dairy cattle, are slaughtered by households or unregistered slaughterhouses. The high percentage of pigs slaughtered outside slaughterhouses is due to the slaughter of pigs by households for their own needs, especially in the colder season, as well as the slaughter of piglets. Of the total number of slaughtered pigs, 30% are piglets slaughtered during religious and national holidays, for celebrations, or for the needs of bakeries and restaurants. Most of the sheep (88%) are slaughtered by households for their own needs. In the case of sheep, 2/3 of lambs are slaughtered for the same reasons as piglets (holidays, celebrations and the hospitality trade). According to official data, practically all poultry is slaughtered in slaughterhouses, but this does not correspond to the actual situation, because the number of poultry (broilers) raised in households remains unknown.

The decrease in the rural population and its age structure has contributed to the decreased livestock numbers in Serbia. According to the 2022 census, Serbia had around six million resident inhabitants. The population decrease for 2011 was −37,337 from the previous census, and for 2021, was −74,442 inhabitants. The average age of residents in Serbia in 2011 and 2021 was 40.2 and 43.5 years, respectively.

5. Slaughterhouse arrangement

Slaughterhouses and the meat industry are among the oldest and most complex systems in the modern food industry, given the fact that they produce a nutritionally valuable food for human consumption, and that the food must be safe for consumption and must not negatively affect the health of consumers. The meat industry is engaged in slaughtering animals, processing meat into various products, disposing of by-products (e.g., skins), purifying waste water and taking care of environmental protection. Slaughterhouses are one of the links in food (meat) production that are vertically connected in a food chain for which the phrase "from field to table" is used. Often in the world, including in Serbia, this chain is owned by one company.

Guidelines on the organisation of slaughterhouses were provided by the Codex Alimentarius Commission in the document, Fundamental Principles of Food Hygiene. These principles have been accepted in over 189 countries worldwide that are members of this body. Among them is the former Yugoslavia, which in 1989 adopted the *Regulation on the conditions that must be met by facilities for slaughtering animals, processing, treatment and storage of products of animal origin* (*Anon*, 2010). At that time, it was one of the best regulations in Europe regulating this area. With amendments from 2008 and 2010, it is still applied in Serbia today. This Regulation refers to the conditions in terms of construction, technical organisation, equipment, working methods, professional staff and hygiene that must be met by slaughterhouses, cold stores and facilities for processing, treatment and storage of products of animal origin intended for public consumption or for export. According to the volume of production, they are divided into industrial, artisanal and household facilities (only honey, milk and eggs). The Regulation defines general and special conditions for the construction and arrangement of the facility. The general conditions relate to the location, circuit, roads and layout of buildings, as well as to water supply (including water supply sources, with hot water at 83°C), wastewater drainage, materials for the construction of premises, equipment, a dedicated room for washing equipment, veterinary inspection, the needs of workers and maintaining the hygiene of the employed staff. Formal facilities for slaughtering animals (slaughterhouses) can be industrial or artisanal. They are divided into these two groups based on the layout and equipment in the facility. The equipment conditions are defined more closely for each room, whereby the technological connection, number and size of rooms and the equipment they contain must correspond to the type and volume of production. After veterinary inspection, carcasses and edible organs of slaughtered animals are transported to a meat cooling room. The size of the room, or rather its cooling capacity, determines the slaughter capacity (volume) expressed in the number of animals per hour or per day (Anon 2010).

Work in a slaughterhouse not only requires skill and strength, but is also made difficult by working conditions (humid air, slippery floors and stands, noise, repetition of the same action, injuries from hand tools, etc.). In a slaughterhouse, the work and procedures of a veterinary inspector (official control) are defined by regulations (*Anon*, 2010.).

6. Mobile slaughterhouses

The first mobile slaughterhouses were used in Great Britain (in the 1960s) for carcass processing (skinning, evisceration, dismemberment) of hunted deer. Thirty years later, mobile slaughterhouses for pigs, ostriches and poultry appeared (*Romero*, 2021; *SANMO*, 1998). Most often, mobile slaughterhouses are used for on-farm slaughter of those dairy cows that have been taken out of production, which are difficult to move, are unstable on their feet, and for which any effort to move poses a risk of falling, bone fractures, and the inability to get up again without human help. Therefore, lorry transport of these animals affects their welfare and exposes them to stress, which consequently, affects the quality of their meat.

In fact, from their place of residence to the stun box, animals destined for slaughter are constantly exposed to stress because they are in environments and situations they have not been in before (loading ramp, means of transport, transport, unloading, stay in the lairage, corridor to the stun box, the stun box itself, stunning). Any separation from a known environment or from a known group is stressful for the animal. Unsuitable loading ramps, i.e., steeply sloped and and without cross bars or slippery, can create stress in the animals and also the risk of injury (joint

dislocations, limb and rib fractures). This especially applies to dairy cows that were previously housed constantly in a confined space with limited movement possibilities, and are unaccustomed to stress; sometimes these animals are in poor condition (*Eriksen et al*., 2013; *Astruc and Terlouw*, 2023). The conditions under which transport takes place are defined by European Directive 2005/1/EC (*EC*, 2005). The aforementioned directive states "no animals shall be transported unless it is fit for the intended journey, and shall be transported in conditions guaranteed not to cause them injury or unnecessary suffering". The animals must not be transported if "they are unable to move independently without pain". If the animal cannot be transported due to the above reasons, it must be treated or euthanised. The competent veterinary inspector assesses the animal's ability to be transported, while the veterinary decision on transport also partly depends on how long the transport takes. When slaughtering in mobile slaughterhouses, there are much fewer, indeed minimal, stressful factors (*Carlsoon et al*., 2004; *Ursinus et al*. 2023).

In several countries (developed, developing and underdeveloped), mobile slaughterhouses are moved (transported by lorries) from one place to another as needed. These are most often two modified shipping containers that are placed in appropriate locations so they can be connected by their shorter sides, thus forming a single unit. In the first half of the first container, the animals are slaughtered, the lower parts of the legs, horns and skin are cut off (the unclean parts), while in the second half, the carcass is eviscerated, and the organs and carcass are inspected. The openings on the sides of the container are used to eject inedible parts into suitable receptacles. The first two-thirds of the second container is the refrigerated cooling area, while the last third is used to prepare the unloading of cooled carcasses and their loading into a refrigerated transport in which the cooled carcasses are shipped to wholesale or retail centres (*Ljungberg et al*., 2007). Mobile slaughterhouses usually have a capacity of 40 to 60 lambs (sheep) per day. These slaughterhouses can ensure good hygienic conditions for slaughtering and processing carcasses, cooling carcasses, waste disposal and sorting of risk material (for sheep older than one year, brain, spinal cord, eyes, etc.) as provided for by the regulations for the protection against transmissible spongiform encephalopathies. In the United Kingdom, mobile slaughterhouses meet the conditions regulated by Regulation EC/2004, they are licensed, and information about them can be found on the Food Standards Agency website. Other countries in which mobile slaughterhouses are used have similar regulations. Before setting up a mobile slaughterhouse at the desired location, it is necessary to provide a room (depot) for lairaging animals before slaughter, a corridor and a ramp connecting the depot and the slaughter area in the container, electricity connections (the largest consumption level is from the refrigerated cooling system), sufficient water (100 litres per sheep), waste water disposal and treatment, disposal of inedible parts and confiscated items, and especially, handling of hazardous materials. The International Patent Commission registered a patent in 2015 for amobile slaughterhouse for slaughtering sheep and goats. The patent is registered with the World Intellectual Property Organization, which consists of more than 130 countries around the world. Today, a large number of companies worldwide are engaged in the production of mobile slaughterhouses, and information about them and their products can be found on the Internet (*Hoeksma et al.*, 2017).

7. Advantages of mobile slaughterhouses

The justifications for building small, mobile slaughterhouses are most often related to satisfying animal welfare and meat quality requirements, reducing dependence on food imports, strengthening local communities and supplying them with quality and safe food, increasing the profits of small producers, ensuring local producers become a more vital part of the food economy, establishing direct links between consumers and farmers, and encouraging organic production (*Ljungberg*, 2007; *Njisane and Muchenje*, 2013; *Hoeksma et al.*, 2017).

Today, mobile slaughterhouses are used for slaughtering ruminants (cattle, sheep, goats), pigs and poultry. Animal slaughter capacities in mobile slaughterhouses are usually 10 to 15 cattle per day, 15 to 20 pigs, or 10 to 50 small ruminants (*Anon*, 2015).

The maximum duration of transport generally can be up to 8 hours, but the actual transport duration depends on the type of animal, age, climatic conditions, density of animals in the vehicle, supply of water and food, condition of the roads, training of the driver and the procedure of the workers. Horses and pigs can withstand the longest transport (up to 24 hours). The transport of cattle and sheep should not last longer than 14 hours continuously. Exceptions are suckling lambs, which meet their water needs by sucking and have not been taught to drink water. Therefore, their transport must not last longer than 10 hours (*Carlsson et al*., 2004).

Animal welfare can be impaired by lack of food and water, lack of suitable flooring, overnight confinement, mixing of groups, separation from the group, insufficient ventilation, shearing of sheep before slaughter, illegal handling (e.g., pulling by the wool for movement, hitting animals) and improper stunning or bleeding. Proper transport and handling of animals before slaughter and slaughter, including in mobile slaughterhouses, are generally important factors for animal welfare that can affect production results and consumer preferences when choosing meat. According to previous surveys, consumers who appreciate animal welfare are ready to pay a higher price for meat from animals slaughtered in mobile slaughterhouses. However, in addition to welfare, long animal transport can negatively affect meat quality parameters (pH, colour, texture, sensory properties, ability to bind water, the presence of bruising) (*Astruc and Terlouw*, 2023; *Ursinus et al.,* 2023). Naturally, mobile slaughterhouses can have an advantage with regard to these aspects over traditional, stationary slaughterhouses.

One of the parameters of the application of mobile slaughterhouses is the cost of carcass processing, about which there are few data. The costs of broiler slaughter and carcass processing in mobile slaughterhouses and small communal slaughterhouses on poultry farms are identical, but are also higher than when broilers are slaughtered in large slaughterhouses. Naturally, the profit generated by mobile slaughter depends on numerous factors (price of mobile slaughterhouses, number of slaughtered animals per time period, price of the finished product, energy costs, water consumption) (*Angioloni et al.*, 2015).

Mobile slaughterhouses in Serbia would certainly contribute to reducing the number of animals slaughtered in households and unregistered facilities. This especially applies to lambs and piglets, which are usually slaughtered without veterinary supervision. The number of poultry slaughtered outside slaughterhouses is a complete unknown, but certainly is significantly higher than is shown in the statistical data. Lambs slaughtered in registered facilities are usually intended for export, as this requires assurance that the lambs come from a registered facility. The use of mobile slaughterhouses, in addition to reducing the number of animals slaughtered without veterinary supervision, would probably result in increased numbers of sheep raised in the hilly and mountainous areas of Eastern and Western Serbia. Producers (herders) would have direct contact with the mobile slaughterhouse and there would be no intermediaries (buyers) between these two parties. Consequently, the producers would have higher profits, and the butchers would be able to place better quality meat on the market. Indeed, the idea of building a stationary small ruminant slaughterhouse in central Serbia is less desirable, even though animals from all over the country, primarily lambs, would be slaughtered. This would necessitate the need for organised purchase, collection of animals in specified places, mixing of animals of different origins, and transport to the slaughterhouse. This would certainly affect animal welfare more negatively, due to the extended time from purchase to slaughter, than if mobile slaughterhouses are used (*Alvseike et al.,* 2019; *Križman and Dobeic*, 2023).

8. Slaughter of animals for military purposes

Mobile slaughterhouses used by the military are portable and equipped to work in emergency conditions. The Serbian Army has, in wartime, during manoeuvres and for army training in field conditions, the ability to slaughter animals, process carcasses, cool meat, and transport it to the army kitchen for meal preparation. In an 8-hour period, one military mobile slaughterhouse (run by a specialised butchery platoon) can produce two tons of meat, depending on species and size of animals. The equipment necessary for animal slaughter and carcass processing is described in the army quartermaster's manual of material resources. The basic part of the butchery platoon's set is a tent with two departments: (i) animal slaughter, carcass evisceration and processing and (ii) carcass cutting. The set includes the means and equipment for receiving and lairaging animals, slaughtering the stock, and cooling and distributing meat, and the equipment and means for maintaining hygiene. The tools and accessories belonging to the butchery platoon are specifically determined and kept separate from other platoons' equipment. The butchery platoon also has dedicated transport vehicles (refrigerator, water tank, trailers) and a prescribed method to pack the equipment. A butchery platoon consists of a group of soldiers and officers in which there are two butchery departments, drivers, procurers, a veterinary technician and a platoon commander (most often a veterinarian). The duties and manner of work are prescribed for each member of the platoon. The details specified for animal slaughter in field conditions relate to knowing the species and age category of the animals, their transport and reception; veterinary inspection and animal holding facilities before slaughter

are described in particular. The processes of animal slaughter and carcass processing (skinning and evisceration), carcass cutting and meat processing are also all described. Veterinary examination of the organs and carcasses of slaughtered animals, including trichinoscopy in the case of pigs and ungulates, is mandatory. The prescribed meat marking method differentiates the meat into the categories of fit for human consumption, conditionally fit and unfit for human consumption. The organisation of animal slaughter animals under field conditions and during war is also briefly described (*Janošević et al.*, 2017).

9. Conclusion

Mobile slaughterhouses must meet the specific and general conditions for animal slaughterhouses, which are already prescribed by regulation in Serbia. In order to obtain safe meat, it is necessary to respect modern principles of meat production, which include the application of good manufacturing practices, good hygiene practices, standard operating procedures and the HACCP system. Animal slaughter and carcass processing, i.e., the entire production process, must be under the constant supervision of veterinary inspection.

Mobile slaughterhouses, especially in the hilly and mountainous parts of Serbia, would contribute to the improvement of livestock production, especially for the sheep sector. The use of these slaughterhouses in the food production chain would contribute to better and stronger connection of all chain participants. The location and number of mobile slaughterhouses needs to be based on appropriate knowledge and consideration of the raw material inputs, the state of livestock production and human resources in districts, and even of municipalities or groups of nearby municipalities in areas within Serbia.

Da li su Srbiji potrebne mobilne klanice?

Milan Z. Baltić, Marija Starčević, Ivana Branković Lazić, Milica Laudanović, Nataša Glamočlija, Boris Mrdović i Vesna Đorđević

INFORMACIJE O RADU

Ključne reči:
Klanje životinja
Transport
Dobrobit
Mali preživari
Potrošači

APSTRAKT

Mobile klanice prvi put se pominju 1960. godine a koristile su se za obradu trupova odstreljene jelensake divljači. Danas se njihova upotreba za klanje životinja, obradu trupa i hlađenje mesa odnosi, pre svega, za klanje farmskih životinja (izlučenih muznih krava) koje se otežano kreću i teško podnose duži transport kao i za klanje jagnjadi sisančadi, koja nisu načena da piju vodu a transport je duži od 10 sati. Najčešće se upotreba mobilnih klanica vezuje za dobrobit životinja, odnosno brojne stresne situacije kroz koje prolaze životinje od mesta gajenja do boksa za omamljivanja, a zatim iza kvalitet mesa. Za dobrobit životinja naročito su zainteresovani potrošači koji su spremni da plate veću cenu mesa dobijenog od životinja koje su bile izložene manjem stresu (dug transport, nedostatak hrane i vode, prenatrpanost vozila, vremenski uslovi). Prednosti mobilnih klanica je pored smanjnja dužine transporta i direktna veza između odgajivača i klaničara (nema posrednika, otkupljivača). Ovo je od posebnog značaja za brdsko-planinska područja Srbije u kojima se gaje mali preživari, uglavnom u seljačkim domaćinstvima, sa mnjim brojem životinja. Upotreba mobilnih klanica uticala bi i na smanjenje broja životinja zaklanih u domaćinstvima (mali preživari, prasad) i van veterinarskog nadzora. Za primenu mobilnih klanica neophodno je dobro poznavanje sirovinske baze (vrsta i broj životinja, obim proizvodnje hrane za životinje) demografski podaci, saobraćajnice, energija, vodni resursi itd. Mobilne klanice moraju da ispunjavaju sve uslove za rad koji se odnose na stacionarne klanice.

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Review paper

Determination and implementation of traceability tools for the meat and meat products supply chain to promote consumer awareness and public confidence

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ABSTRACT

Governments have focused on the design of tracking systems due to concerns about the security of imported foods and prevention of zoonotic diseases. The required infrastructure, data collection methods, and health benefits and components achieved through the implementation of traceability at the international level were reviewed and reported in the present study. The review demonstrated that the implementation of each electronic tracking system allows the identification of consumed meat from farm-to-fork. However, the Radio Frequency Identification (RFID) systems, DNA markers, and Wireless Sensor Networks (WSNs) were indicated as the most appropriate and accurate methods for tracking the origins of consumed meat. According to our findings, regulatory bodies and policymakers need to pay robust attention to this issue to prevent the penetration of counterfeit meat products and to maintain general public health.

1. Introduction

Food traceability is a preventive approach for creating and maintaining an information path that tracks a product's movement throughout the production process to ensure the origin of the food product (*Bougdira et al.*, 2019; *Ghag and Shedage*, 2025). Following outbreaks of zoonotic diseases and human health concerns, tracking systems were introduced for the meat supply chain (SC) (*Levings,* 2012; *El-Sayed et al.*, 2016; *Zhao et al.*, 2020). Animal identification is the basis of tracking systems in the meat SC, in which records of an animal are documented from its birth to slaughter, as is the supply of its meat to the consumer (*Zhao et al*., 2020; *Singh et al.*, 2025).

One of the fundamental steps in tracking is food labeling. Although this does not provide traceability *per se*, it is an important part of the tracking policy that allows for physical tracking of the product and can be used as an effective tool for product differentiation and quality affirmation (*Alfian et al*., 2017; *Fan et al*., 2024). In this regard, the European Food Safety Act (178/2002) and the European Beef Hygiene Act (1760/2000) specify that meat labels should contain the following mandatory information: 1) reference number for matching the slaughtered animal and its meat; 2) countries of the animal's birth, raising, and slaughter; 3) country/countries in which meat was fragmented, and; 4) slaughterhouse(s)' identity numbers. Optional information includes animal breed, the type of diet consumed, name of the owner(s), vaccination, transport ID, halal/non-halal slaughter, and other components that are written on the meatpacking box when leaving the slaughterhouse, based on each

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country's regulations (*Cheraghi Saray and Rafat,* 2016). However, tags can be easily counterfeited if they are paper-based and use one-dimensional (1D) barcodes without reference to a central database (*Li et al.*, 2024). Therefore, researchers are focusing on other tracking systems that produce a very low possibility of counterfeit and fraud (*Deng and Feng*, 2021).

Two-dimensional (2D) barcodes (in particular quick response (QR) codes) have many advantages over 1D and linear barcodes and are a successful and relatively easy tracing method for consumers to use (*Li et al*., 2024). These barcode scans relay the product information recorded in a central database, such as texts, photos, and videos, to customers who scan the codes with a camera lens, typically in a smartphone (*Chen et al*., 2020; *Li et al*., 2024).

Another new tracking method is radio frequency identification (RFID), which can track and monitor objects in different SCs. To this end, a carcass or a container carrying the carcass can be labeled and attached to an RFID at the time of slaughter when the head, skin, and intestines are separated from the body, but there are no physical body parts for the identification of an animal source (*Yan et al*., 2018; *Ismail and Huda*., 2024). However, the identification (ID) of a slaughtered animal and the exclusive number of the slaughterhouse must also be linked together to allow for tracing. Under these conditions, RFID can connect the sensors and act as a detection black box for tracking, logistics, and anti-counterfeiting purposes (*Yiying et al*., 2019; *Qiao et al*., 2023).

Smith et al. (2008) also developed a retinal scanning method to identify animals at birth, weaning, fattening, and entry into the slaughterhouse. Despite the exclusivity of retinal scanning, the identity of meat cuts in the slaughterhouse is questionable with this method. To complete this approach, DNA tracking is another option for identifying composite meat components, such as minced meat and carcass parts of unknown origin *(Hrbek et al*., 2020; *Nastasijevic et al*., 2025). Since the meat has a unique identifier that cannot be manipulated, and due to the inheritability of DNA, this method, along with microsatellite markers, can explicitly prove the origin of meat and meat products by tracking individual cuts of meat (*Zhao et al*., 2017; *Nastasijevic et al*., 2025).

The use of wireless sensor networks (WSNs) is another method used to monitor the temperature and humidity of packaged, perishable meat products within the SC (*Aung and Chang*, 2014; *Gil et al.*, 2025). By integrating RFID and WSNs, the system can track products from origin to slaughter and also provide information on environmental conditions, such as the temperature and humidity of packaged meat from the slaughterhouse to the time of reaching the consumer (*Yan et al*., 2018; *Davoudi et al*., 2024). Given the described techniques and studies, it can be argued that the necessary infrastructure and potential for the implementation of tracking systems now exist in the food industry of most countries. This is because advances in the field of information technology provide the required mechanisms to achieve fast and comprehensive monitoring methods in any country. However, the implementation of tracking systems requires improvement and integration between relevant institutions and the development of standards for the collection and publication of tracking data (*Cheraghi Saray and Rafat*, 2016; *Qian et al*., 2020b; *Ellahi et al*., 2025). Therefore, this review explains the importance of traceability of meat products and describes successful systems adopted at the international level, with the hope that the relevant institutions and organizations in every country approve and adopt the necessary rules and standards to implement national meat/meat product tracking policies that will advance the health and rights of consumers.

Impacts

- Pandemics of infectious and zoonotic diseases over the last few years have increased the food safety concerns of producers and consumers and the need to pay special attention to accurate traceability of animal products and animal health.
- The present review attempts to raise awareness of current developments in the traceability of meat products, animal health and subsequently, better utilization of animal resources, and finally, presents the tracing systems successfully adopted at the international level.
- Review of recent scientific developments showed that modern electronic devices (such as electronic barcodes, DNA markers, RFID, GPS, EPCIS and other biometric sensors) play a vital role in monitoring and solving the problems encountered by meat producers and other actors in the meat supply chain.
- The results of the present study are of considerable significance in terms of public health because accurate implementation of meat tractability systems can mitigate the risk of zoonotic diseases, increase animal health, improve food security, and contribute to enhancement of vital health standards in different countries all over the world.

2. Materials and Methods

2.1 Protocol

Here we present the results of the literature review for past peer-reviewed papers dealing with meat traceability, consumer awareness, public health and related topics. Papers were collected from the CAB Direct, PubMed, Scopus and ISI Web of Science with topics (title, abstract, and author keywords) including different methods of tracking systems and identity of consumed meat source from farm-to-fork. After removing the irrelevant papers, there were 317 fully peer-reviewed papers on this topic between 2000 and 2025 (Table 1). Reference lists in eligible articles and relevant reviews were hand-searched to identify and include further relevant papers. Subsequently, the results of relevant papers were merged, and consensus was reached by discussion among the authors on any disagreements. Finally, due to the wide range of traceability approaches, only the most relevant and frequently reported topics were selected for comparison and discussion.

2.2 EAN.UCC system in traceability

The EAN.UCC system provides internationally recognized standards for the unique identification of food products at all stages of production, transportation, and storage. It also provides facilities for electronic communication standards to enable the accurate and quick exchange of information between all stages of food production, processing, and distribution (*Zhao and Cao*, 2017). The system uniquely identifies products, locations, services, and assets, and includes a set of standard data structures, called Application Identifiers (AIs), which allow encoding of secondary information, such as batch number, expiry date, and other meat resource properties for encryption. The basis of the EAN.UCC system, which is used extensively in traceability, is an unambiguous numbering scheme used to identify goods and services throughout the SC (*Bai et al*., 2017). Owing to the automated techniques for information recording in this system, the numbering method can be used at any stage of production, conversion, and distribution of meat and its products.

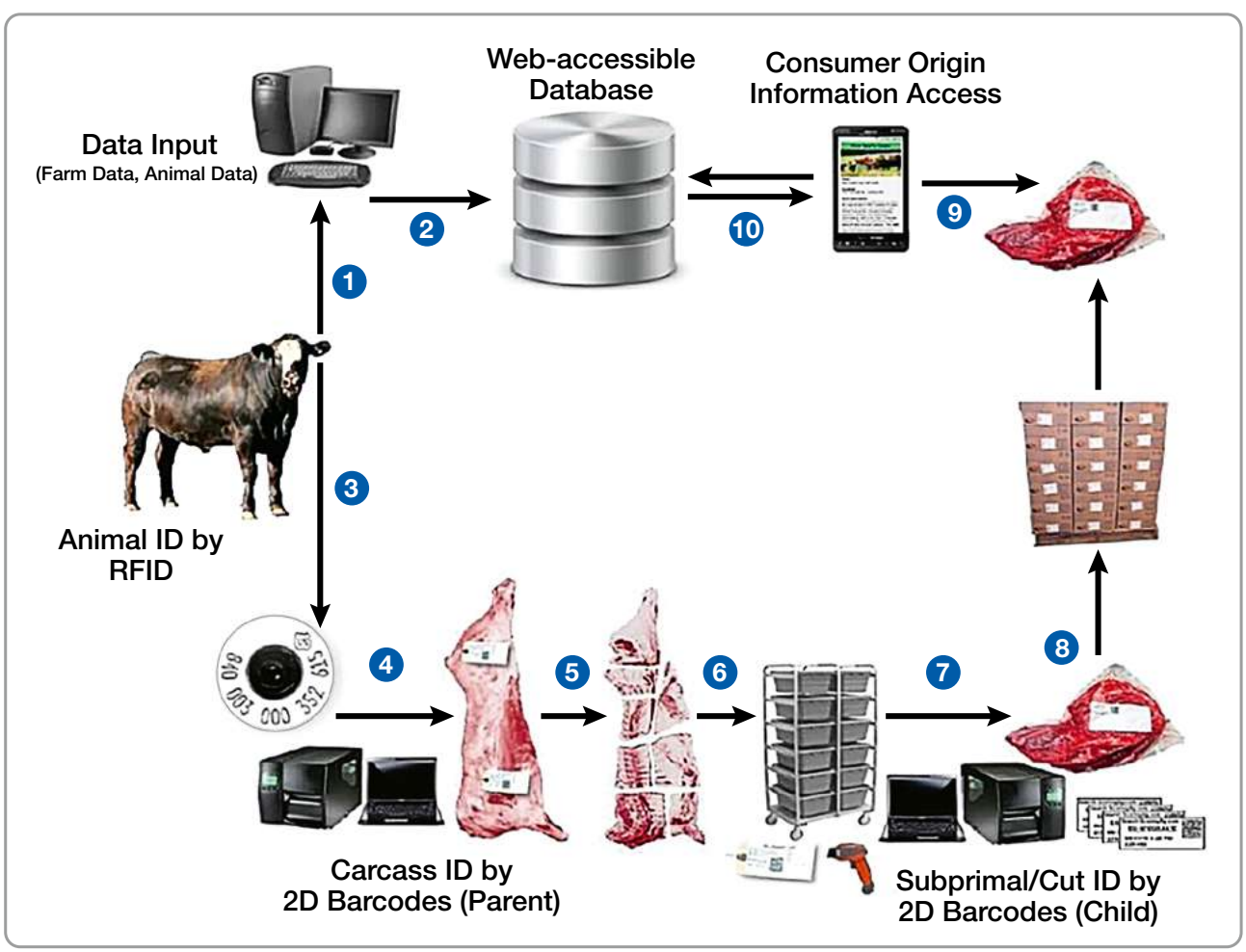


Figure 1. Different stages of meat tracking (adapted from Buskirk et al., 2013)

Table 1. Comprehensive search strategy for articles selection

Database	Searched keywords	Number of results	Number of selected articles
CAB Direct	(animal traceability/ animal health/ meat/ minced meat/ meat sources/ animal muscle composition/ animal tissues/ animal traceability system/ supply chain/ value chain/ supply networks/ consumer awareness/ consumer rights/ right to safety/ consumer protection/ health/ public health/ consumer's health/ zoonotic disease/ animal traceability/ slaughter stage traceability/ post-slaughter traceability/ traceability policies/ government policies/ regulations/ traceability benefit/ traceability benefit problem/ traceability tools/ barcodes/ RFID/ EPCIS/ DNA markers/ genetic traceability)	531	98
PubMed	(animal health/ traceability system/ meat traceability/ slaughterhouse processing/ post-slaughter traceability/ tissues traceability/ muscle composition traceability/ minced meat traceability/ food trace number/ packaging/ zoonotic disease/ meat transportation/ meat distribution industry/ consumer rights/ consumer health/ public health/ food safety/ animal resources)	87	27
Scopus	(meat industry/ meat sources/ meat traceability/ meat supply chain/ meat traceability systems/ meat traceability tools/ blockchain/ barcodes/ two-dimensional (2D) barcodes/ 2D tags/ quick response (QR) code/ radio frequency identification (RFID)/ wireless sensor networks (WSNs)/ electronic product code information services (EPCIS)/ DNA markers/ DNA tracking/ genetic traceability/ tracking technologies/ meat packaging/ freshness indicators/ temperature indicators/ gas indicators/ biosensors indicators/ consumer rights/ consumer awareness/ consumer confidence/ consumer health/ public health/ health benefits/ health concerns/ food safety/ food security/ zoonotic disease/ national policies/ governmental policies/ food safety policy/ meat traceability policies/ traceability benefits/ traceability costs/ animal resources/ animal products)	114	66
Web of Science	(animal product safety/ meat quality/ meat chain control/ meat traceability/ packaging traceability/ meat packaging/ identification/ animal identification/ animal authentication/ animal genetics/ genetic traceability/ DNA microsatellite markers/ animal muscle types/ minced beef/ beef supply chain/ food supply chain/ cold chain/ blockchain/ perishable food supply chain/ food monitoring for safety/ rapid alert system for food and meat/ automotive applications/ online temperature monitoring/ optimal temperature/ shelf life/ electronic pedigree/ labeling/ 2D barcode technology/ QR code/ RFID/ WSNs/ EPCIS/ food safety criteria/ border control/ microbial food safety policy/ governmental policies/ meat traceability policies/ public health/ veterinary public health/ zoonotic disease / food safety / food security / consumer health/ consumer trust/ consumer awareness/ consumer rights / animal resources / animal products / animal health)	217	92
Other Academic Databases	(animal health/ traceability technologies/ food traceability/ meat traceability/ blockchain / meat/ minced meat/ meat sources/ animal identification/ animal authentication/ veterinary public health/ zoonotic disease / food safety / food security / public health/ consumer health/ consumer trust/ online temperature monitoring/ automotive applications/ Mobile solution/ rapid alert system for food and meat/ electronic pedigree/ labeling/ QR code/ 2D barcode/ WSNs/ RFID/ EPCIS/ microbial food safety policy/ meat traceability policies/ national policies/ meat safety policy)	368	34

2.3 Traceability at the slaughter stage

After entry into a slaughterhouse, the identity and history of animals are transferred to and tracked by the slaughterhouse's central database. After slaughter, the skin is removed and the carcass divided into the hindquarters and forequarters. The slaughtered carcass then transfers to the cutting room, and the bony part of the carcass is first separated and each section is turned into primary cuts (*Cheraghi Saray and Rafat*, 2016; *Thakur et al.*, 2020). After preparing the required cuts, vacuum packing is often applied and the meat labelled with a special barcode. The label contains the product code, package code, country of origin, birth date, names and addresses of producers, carcass number, sex and cold weight of the carcass, ear tag number, date of slaughter, country of slaughter, name of slaughterhouse, carcass cutting plant, the date of packing, and expiry date. Finally, the product enters the cold or freezing rooms and a traceable code is recorded for it at the time of transport. At the same time, all information about the post-slaughter time and transport is recorded in the central database (*Buskirk et al*., 2013). A schematic of different stages of meat tracking is shown in Figure 1.

2.4 Post-slaughter traceability

To differentiate the meat of slaughtered animals originating from different feeding systems, a good approach is spectral characterization of the meat using reflective spectroscopy. In this method, the meat muscle type is detected and differentiated using principal component analysis (PCA) and independent modeling, and qualitative analysis of the obtained information determines the difference in meat muscles from two or more different feeding systems (*Horcada et al*., 2020; *Barragan et al*., 2021). Moreover, another approach can detect the meat of a particular animal that is turned into minced meat by simple carcass processing at a specific time (*Spence et al*., 2018). In this method, a single individual scan of the animal is already recorded at the slaughterhouse to preserve its identity. When the animal is slaughtered and divided into primary parts, each one is marked with a special barcode. Each piece of meat receives a unique food trace number before packaging, which links it to a group of animals slaughtered on a particular day. Therefore, using a food trace number, the source of meat is displayed after slaughter and packing and will be shown when final product is sold at butchery counters. Thus, this is a suitable method for tracking meat based on EAN.UCC standards, which is able to track complete or minced meat throughout the SC (*Cheraghi Saray and Rafat*, 2016; *Bai et al*., 2017) (Figure 2).

3. Different tracking tools and systems

3.1 RFID

RFID tags are one of the most effective methods for tracking animals, consisting of a circuit (preservation of a unique identifier number), an antenna (connected to a microchip), and a memory component (allows recording information and

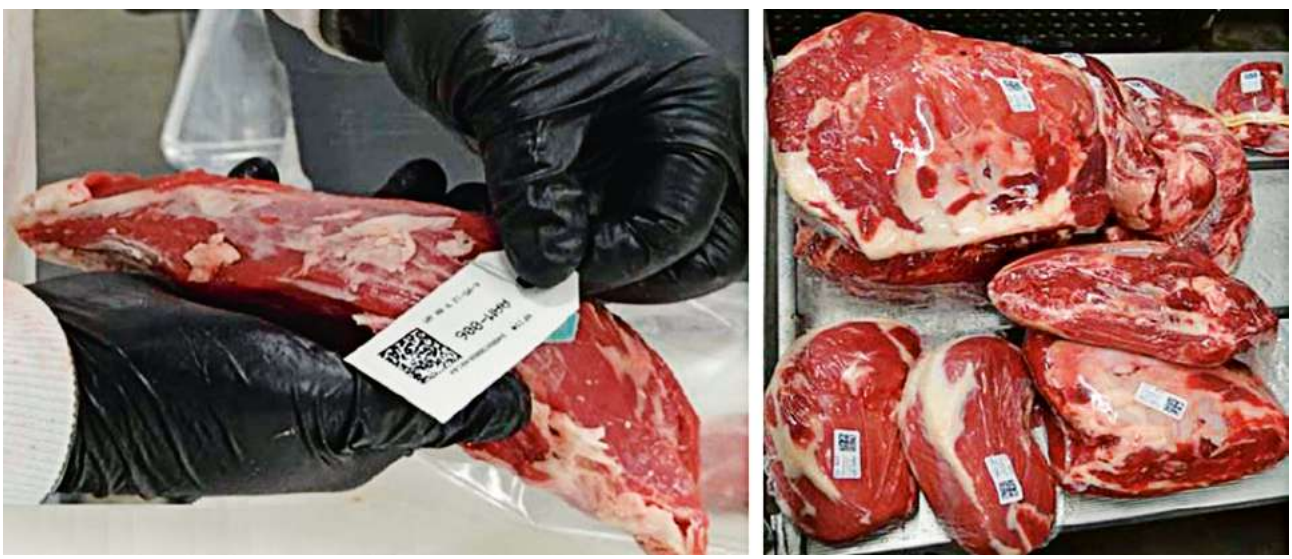


Figure 2. Assignment of food trace number to each cut of meat. The food trace number is a unique reference number for traceable information used on a specific day and time of slaughter. Upon a customer's request about the origin of the meat, the slaughterhouse, processing plant or group of animals that originated there can be traced using this number (*Cheraghi Saray and Rafat*, 2016; *Yiying et al*., 2019).

Figure 3. A sample of RFID tags and their function

communicating with the reader), and all are connected to a computer system (Fig. 3). Radio waves are emitted from the RFID tag, converted into digital data by the operator, and added to the information systems of relevant companies or institutions. Various coatings are used to protect the circuit from dust, extreme temperatures, humidity, heat, and salt. The workable distance that between the tag and the reader or operator depends on the frequency band (*Velandia et al*., 2016; *Zhang et al*., 2017).

3.2 Two-dimensional labels (2D tags)

The QR code is one of the tracking systems that can embed significant information, such as text, video, advertising, personal information, etc., in the form of a 2D barcode. These codes can be easily scanned with smartphones to decrypt information and messages related to the meat products. In this method, even offline users can access the meat product information at any situation, simply by installing a 2D barcode scanning application on their smartphone (*Cheraghi Saray and Rafat*, 2012; *Chen et al*., 2020).

3.3 *DNA markers*

Despite the high cost of measurement methods based on the DNA marker tracking technique, these methods are very effective and have many advantages over paper-based tracking methods. Microsatellite markers can be used to detect the meat breed of an ID-less slaughtered animal (*Zhao et al.*, 2017). The analytical methods used in this method are mainly based on protein and DNA analysis. Protein-based methods include immunological methods, electrophoretic assays, and chromatographic techniques, each of which is measured according to the relevant standards (*Hamishehkar et al*., 2014; *Hrbek et al*., 2020). In general, DNA-based meat source traceability systems mainly follow a similar path. In these systems, tissues, hair follicles, and blood samples from carcasses or live animals are obtained from each animal or carcass before or during slaughter, and DNA analysis results are stored afterward. When the carcass enters the cutting room, any initial or packaged cut is identified as an animal or as within a group of animals that have passed simultaneously through the slaughter stages. When verification is required, the meat or packaging information is connected with the stored materials and with the DNA profiles. Finally, a group of stored DNA profiles, which should contain that from the carcass in question, is selected, and the relevant DNA profile are fully matched to the carcass or the animal group from which it originates (*Shackell*, 2008; *Kademi et al*., 2019; *Qian et al*., 2020a).

3.4 Electronic Product Code Information Services (EPCIS)

The EPCIS is an online system based on monitoring the temperature and humidity in the hot and cold meat SCs. During meat transportation, this system is used by RFID-based temperature sensors to record product temperature and predefined data at any time and place in the transportation chain (*Thakur and Foras*, 2015; *Wang et al*., 2017).

3.5 Other new tracking technologies

Other technologies, such as freshness indicators (estimation of a product's remaining shelf life), temperature indices (indicating temperature history during distribution and storage), gas indicators (monitoring changes in gas composition within packaged containers), and biosensors (detection, recording, and information on biochemical reactions) are new methods known as intelligent tracking for packaged meat products (*Han et al*., 2018). The design of such packages for meat sources and their integration with recording and data transfer devices have enhanced

logistics activities that have a significant effect on the flow of meat sources from farm-to-fork, thereby increasing the efficiency of meat source tracking efforts (*Fang et al*., 2017).

4. Data mining

Some data are expected to be non-recordable for a variety of reasons. Data mining techniques are used to predict and estimate such data throughout the SC to ensure a complete record of meat products. The integration of data mining techniques with tracking systems ensures the quality and safety of meat food sources throughout the SC so the consumer can evaluate and judge the quality of meat products in any situation before purchasing (*Alfian et al*., 2017).

5. Results and Discussion

5.1 Different tracking tools and systems

5.1.1 Genetic traceability (DNA markers)

The results of review studies have shown that genetic traceability can play a very important role in food chains, because genetic traceability is a rapidly growing application due to the rapid development of genomics, not only in food identification but even in the control of nutrition (Cheraghi Saray and *Hosseinkhani*, 2013; *Qian et al*., 2020a). According to *Morcia et al.* (2016), DNA is a stable molecule that exists in all types of tissues and can retain sequence-specific information that can be accessed by a simple replication reaction. Therefore, next-generation sequencing technologies are able to produce large amounts of genetic data in a short time at a reasonable cost (*Ghosh et al*., 2018; *Zhao et al*., 2019). In addition, it has been shown that the nucleus genome could be identified for individual animal species by extracting information from genetic sequences. Accordingly, stability measurements could be designed for detection purposes and generally to characterize each animal, plant, and microorganism (*Romanenko*, 2017; *Hrbek et al*., 2020). Similar results have confirmed that the DNA marker technique is a suitable method for determining the origin of an animal meat sample (*Zhao et al*., 2017). This method requires using at least eight molecular markers that provide a high degree of mean heterozygosity in a population, so achieving unique identification of individuals in the population (*De-Camargo*, 2018). Currently, several classes of PCR-based DNA markers, along with direct sequence analysis, have been used frequently to identify plants and animals involved in the human food chain (*Morcia et al*., 2016). In addition to high accuracy, DNA marker traceability is a relatively simple technique, so these tests could enhance knowledge of

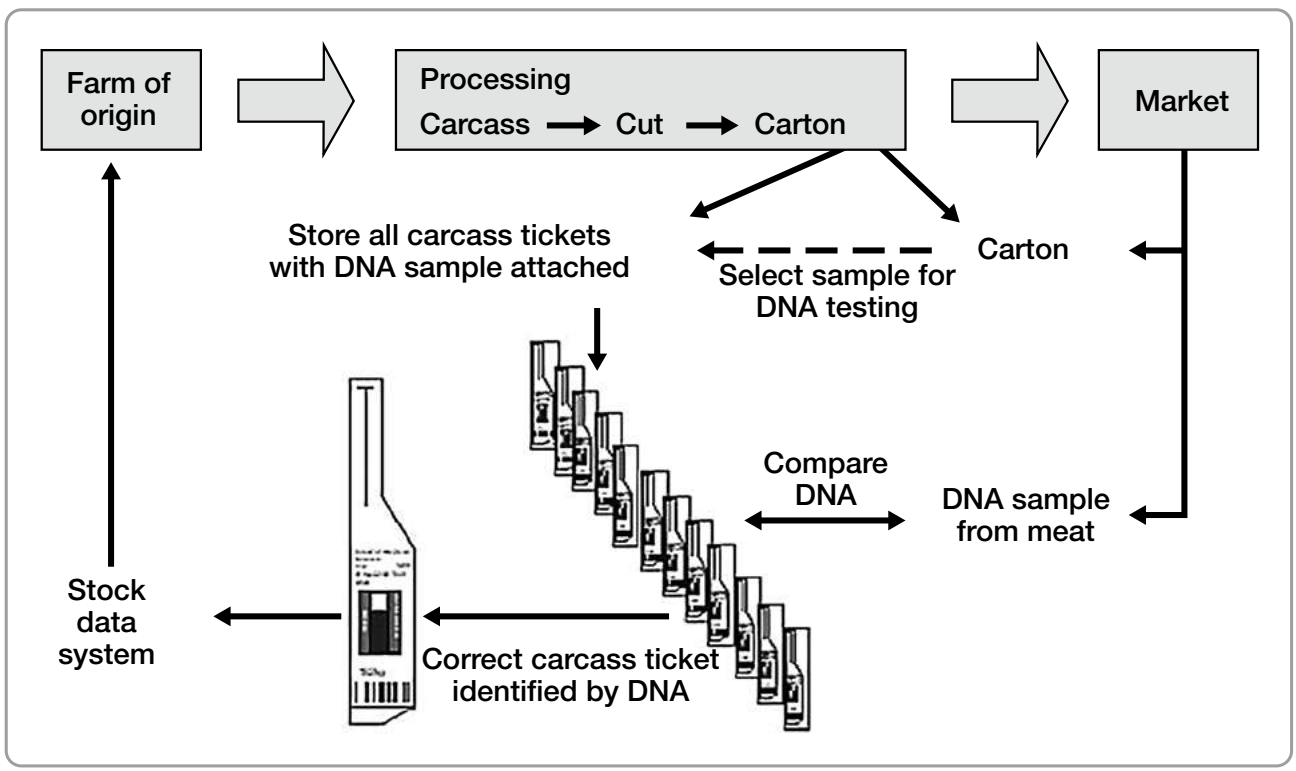


Figure 4. The general path of DNA-based meat traceability

the quality attributes of produced meat and increase consumer confidence (*Zhao et al*., 2019). DNA-based meat source traceability systems mostly follow the same general path as shown in Figure 4. It is noteworthy that the successful implementation of this method requires a basic knowledge of population structures related to meat food sources. Although the accuracy of DNA-based traceability steps is almost completely guaranteed, its main limitation is the cost. However, systems for counterfeit prevention strategies can be implemented at lower costs through national and international certification of meat sources (*Cheraghi Saray et al*., 2019; *Qian et al.*, 2020b; *Cao et al.*, 2021).

DNA-based traceability systems generally follow a common pathway. These systems typically utilize tissue samples, blood from a live animal, blood from a slaughtered carcass, or hair follicles from a live animal. Prior to slaughter (or during the process), a blood or tissue sample is taken from each live animal or slaughtered carcass and held in storage. When the carcass is transferred to the cutting room, each primal cut or packaged portion is identified in a manner that allows for the identification of the individual animal, or a group of animals processed concurrently through the slaughter facility. When a trace is required, a sample of the meat or its packaging information is sent to the DNA sample storage. In this storage, the sample can be unambiguously matched to the carcass or animal from which it originated, using DNA profiles. For any DNA-based system to be effective, a traceable production pathway through the processing facility is essential. Processors must adhere to standard operating procedures to prevent contamination. When these conditions are met, a validated and standardized analytical method can be employed, facilitating the matching of DNA profiles obtained from the carcass at the time of slaughter with samples of the packaged meat (*Shackel*l, 2008; *Cheraghi Saray and Rafat*, 2016).

5.1.2 RFID

Studies on RFID as one of the tracking system tools revealed that the use of RFIDs has grown along with the development and production of modern electronic devices that can be installed on the animal's ear, under the neck or ankle skin, or when placed in a protective layer, inside the animal's digestive tract (Figure. 5). Small RFID tags in different MHz and GHz frequency bands ensure system integrity and information (Zhao et al., 2020). The use of RFIDs increases both consumer confidence and, in addition to security and control of total production, enhances efficiency. This greatly reduces the system workload and can improve the development rate in addition to facilitating access to network services (Alfian et al., 2017). More sophisticated biometric technologies are becoming more sophisticated for living animals. Therefore, automated tracking systems and RFID-based tracking systems are currently available in many industries. However, RFID technology is expensive, and the high costs are to pay operators, install computer software, provide networks, and maintain related systems (Zhang et al., 2017; Alfian et al., 2020; Urbano et al., 2020). Therefore, the use of RFID is recommended only for companies or organizations that have economically evaluated and justified it. Figure 5 shows an example of the use of RFID in live animals before and after transport to the slaughterhouse.

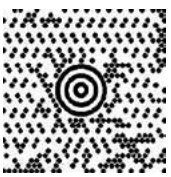
Figure 5. a) Application of RFID in animals from birth and throughout the breeding period until arrival at the slaughterhouse and sending their meat to stores; **b)** Different locations for the installation and use of RFID tags in a live animal

5.1.3 Two-dimensional tags

Two-dimensional barcodes can store a large amount of information as a machine-readable pattern in black and white lines. These barcodes can act as a portable database that is scanned and decrypted by smartphones (*Chen et al*., 2020). The information embedded in this type of barcode is mainly obtained by translating and transferring the information placed in the RFID ear tag to a 2D barcode, which is first embedded on the carcass and then on each piece of packaged meat, and finally provided to the consumer. Hence, the transfer of RFID data to 2D tags leads to 100% accuracy in tracking meat through the SC (*Foster et al*., 2018; *Thakur et al*., 2020). A wide range of 2D barcodes are available, but the four most important examples described in the present study are shown in Table 2. The advantages of the 2D QR code tag have led to its widespread use in the food industry, particularly in the meat industry (*Gao et al*., 2009; *Kim and Woo*, 2016; *Focardi et al*., 2018). The QR code can be a useful tool for implementation of consumer rights by providing more information on food safety and quality. The QR code is also expected to be used more than ever to help eliminate consumers' distrust and strengthen their satisfaction when shopping. Therefore, comprehensive and accurate information, such as the nature, brand, origin, packaging quality, price, safety, stability, and environmental effects of the product, should be provided for each food product. These efforts are important mechanisms that can improve the consumer's decision to buy meat foods (*Kim and Woo*, 2016; *Zhang et al*., 2020). Figure 6 shows an example of the information embedded in a 2D barcode after being scanned by a smartphone. More extensive and accurate presentation of this information will satisfy the consumer and support the product sale.

Figure 6. Information embedded in a 2D barcode when scanning by the consumer

Table 2. Specifications of index samples for 2D barcodes (capacity features and standards for major 2D barcodes[a])

	QR Code	PDF417	Data Matrix	Maxi Code
Example code				
Developer (country)	DENSO (Japan)	Symbol Technologies (USA)	RVSI Acuity CiMatrix (USA)	UPS (USA)
Numeric	7,089	2,710	3,116	138
Alphanumeric	4,296	1,850	2,355	93
Binary	2,953	1,018	1,556	-
Features	Large capacity Small printout size High-speed scan	Large capacity	Small printout size	High-speed scan
Standards	AIM International JIS ISO	AIM International ISO	AIM International ISO	AIM International ISO

Legend: [a]Adapted from Gao et al. (2009).

Table 3. Transportation requirements for the perishable food products

Product	Temperature (°C)	Humidity	Other requirements
Cooked food	> 60–63 (hot holding temperature)		
Chilled food	0–4 (temperatures higher than 4°C cause faster growth of bacteria)		
Frozen food	≤ −18 (temperatures lower than –18 °C prevent bacteria growth)		
Fresh fruits and vegetables	0–8	90%–95%	Appropriate concentrations of O_2, He, CO_2, and C_2H_4

Note: The temperature requirements for food transportation can vary in different countries depending on their regulations (Farooq et al., 2016)

5.1.4 Electronic product code information services (EPCIS)

One of the policies developed for traceability systems is the design of general applications (e.g. applications that can be used in the smartphone) to enable consumer monitoring of food quality and to prevent the penetration of counterfeit products into food SCs. Most perishable food products, including cooked, chilled and frozen meats, require special storage conditions (Table 3), the full details of which are provided to consumers through designed applications (*Hamishehkar et al*., 2015; *Farooq et al*., 2016; *Wang et al*., 2017).

Among the literature reviewed in the present study, an example of EPCIS (Figure 7) can illustrate

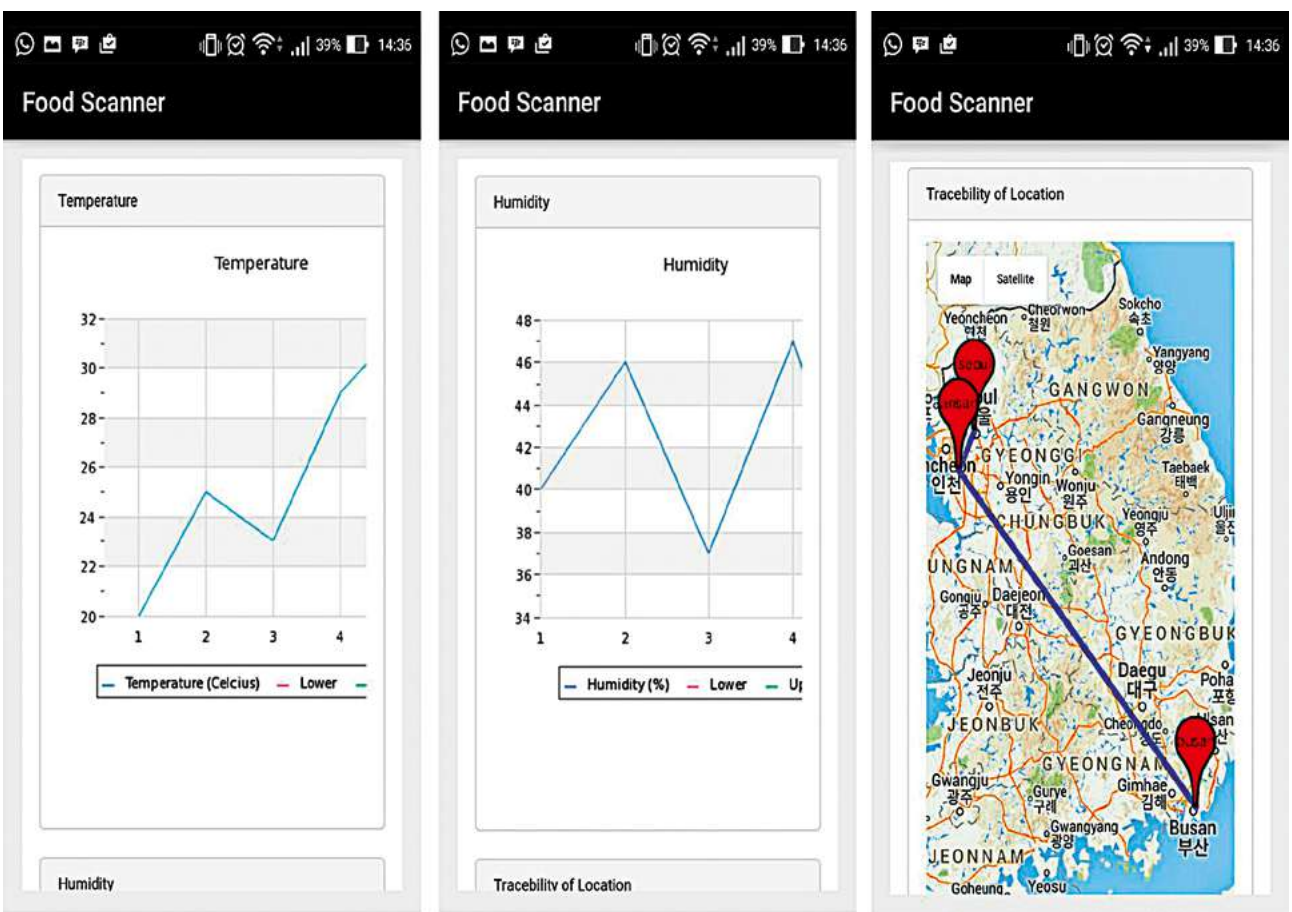


Figure 7. Screenshot of temperature, humidity, and map indicating meat tracking in a smartphone application (adapted from *Farooq et al*., 2016)

the performance of these applications. For instance, the consumer, after installing the smartphone application and scanning the product barcode at the time of purchase, observes graphics showing product temperature and humidity recorded along the depicted route map from the time of leaving the slaughterhouse to arrival at the store. The various pages of this application can graphically compare the product humidity and temperature data with the ideal temperature and humidity data for that product in the SC, and users can ascertain the actual transit route and the duration for which the product has undergone temperature and humidity fluctuations from the slaughterhouse to the store. Ultimately, the consumer is responsible for the final judgment and purchase decision; the data enable sensible discussion about the quality of transportation and the desirability of product transfer (*Thakur and Foras*, 2015; *Farooq et al*., 2016; *Wang et al*., 2017).

5.1.5 Traceability of animal muscle composition and tissues

Studies on tracking different meat sources with a focus on consumer demand to monitor the animals' diets and their meat production indicate that diets can be tracked for meat sources and raw milk using analytical methods. Dietary indicators are determined quantitatively or indirectly from the product or tissues of the slaughtered animal (*Zhao et al*., 2020). One study demonstrated that combined administration of different trackers would be useful, due to differences in costs and ease of implementation among different tracking methods (*Alfian et al.*, 2017). Findings on tracking muscle composition for animal meat raised for live weight gain and fed concentrate feeds due to a lack of forage in pastures revealed that the relative live weight gain using concentrates was associated with stronger changes in isotope C composition (*Monahan et al*., 2018; *Prache et al*., 2020). Also, some isotope C from the previous grazing period still remained in muscles even after 230 days of fattening. This relationship was not observed in their adipose tissues, which was attributed to relatively late fat deposition during the fattening period (*Prache et al*., 2005). In other similar studies, researchers could characterize meat spectra using visible-infrared spectroscopic detectors to differentiate beef that originated from different feeding systems. Their studies demonstrated differences in muscles and fleshy tissues from different nutritional systems by the use of PCA, independent modeling methods, and finally qualitative analysis of optical information (*Horcada et al*., 2020; *Dumalisile et al*., 2020; *Barragan et al.*, 2021). The benefits of this type of tracking become even more important when consumers demand accurate information about the diet type and composition of slaughtered animals (*Hosseinkhani et al*., 2007; *Cheraghi Saray and Rafat*, 2016). Overall, the findings emphasize the fact that, for both meat and milk of a studied animal, the combined use of different tracers can be useful to detect the composition of different tissues or index compounds in specific sediments where forage or feed is grown (*Zhao et al.*, 2020). Accordingly, the combined administration of different tracers and examination of different tissues could improve our ability to predict and monitor the traceability of different meat sources.

5.1.6 Traceability of minced meat

Studies conducted over the last two decades show that, despite many endeavors in accurate traceability, minced meat or animal-derived products were usually exempt from full traceability. This weakness is mainly due to problems in determining and tracking the history of slaughtered animals, which contributes to the lack of accurate tracking of a mixed product (*Salih*, 2017; *Thesmar and Stevens*, 2019). For example, *Heaton et al.* (2005) reported that 9.5% of packaged liver and minced meat portions did not match the animals whose identities were recorded when entering the slaughterhouse. Similarly, other studies indicate tracing violations were committed mainly before the product entry into the processing plant (*Qian et al*., 2020b). However, in recent years and in most countries, data collected for minced meat traceability have been limited to the production date and place of the final production (*Han et al.*, 2018; *Spence et al*., 2018). In this regard, researchers investigated a tracking technique based on DNA to separate the different parts of minced meat, and concluded that the physical separation of the compound ingredients might be the basis for the traceability within products (*Naveena et al*., 2018; Hrbek et al., 2020). In their study (*Naveena et al.*, 2018), the DNA-based identification method could differentiate different compound meat products. Other chemical technologies, such as enzyme-linked immunosorbent assay (ELISA), were able to detect species abnormalities in meat products (*Li et al*., 2019). Despite the sufficient knowledge about traceability and accurate identification of minced meat, conventional tests to accurately

identify minced meat inputs are lacking (*Cherghi Saray and Rafat*, 2016; Salih, 2017). This can be explained by the fact that although accurate and on-time data collection is one of the priorities in the food (meat) SC, the main object of a traceability system is finding the best technology in order to reduce costs, risks, time, and energy expended to provide exact information about product transportation in the food SC (*Galvez et al*., 2018; *Thakur et al*., 2020). Moreover, tracing animal tissues is increasingly difficult given elapsed time after slaughter; this is due to the complexity of handling, equipment, and information requirements that need to be imposed for extensive tracking (*Bai et al*., 2017; *Horcada et al*., 2020; *Barragan et al*., 2021). Therefore, it is reasonable that the inefficiency of minced meat-related tracking in recent years is primarily due to the absence of low-cost, simple, and convenient technologies, and secondly, a lack of consumer concern about their rights to know the origin and identity of mixed meats (*Salih*, 2017; *Spence et al*., 2018). Therefore, governments need to be convinced to adopt appropriate policies to reduce the cost of authentication technologies and tests for the detection of violations and counterfeits committed in the minced meat SC. This requires raising people's awareness and knowledge in this field and increasing their demand for tracking mixed and derived meats, which will constitute a considerable portion of the market for meat food sources (*Cheraghi Saray and Rafat*, 2016).

5.1.7 Traceability in the transportation industry and distribution of meat

Findings related to traceability in meat transportation and distribution industries suggested that the origin of meat spoilage is transmission of bacteria from one animal to another during the slaughter process or at any stage of the production, processing, and meat distribution (*Cheraghi Saray et al*., 2014; *Zare et al*., 2014; *Odeyemi et al*., 2020). *Galvez et al*. (2018) demonstrated that tracking can be a tool for the successful identification, elimination of inappropriately contaminated products from the market, and for supporting product quality assurance processes. Therefore, the implementation of tracking for animals selected for slaughter might have a major contribution in reducing the identification costs of non-standard meat products (*Zare et al*., 2016; *Yan et al*., 2018; *Zhao et al*., 2020). With respect to pathogens, a study by Buhr (2003) is an excellent reference for recent research topics. This researcher reported that the veterinary services of one company identified *Salmonella* in routine tests on animal farms. Through traceability information systems, they proved in the shortest possible time that the *Salmonella* originated from the raising farm, and the need to recall feed, which could have contained the pathogen, was obviated. Economic analysis found that the use of traceability in this situation resulted in saving more than $100,000 in feed recalls (*Buhr*, 2003). Figure 8 shows a simple

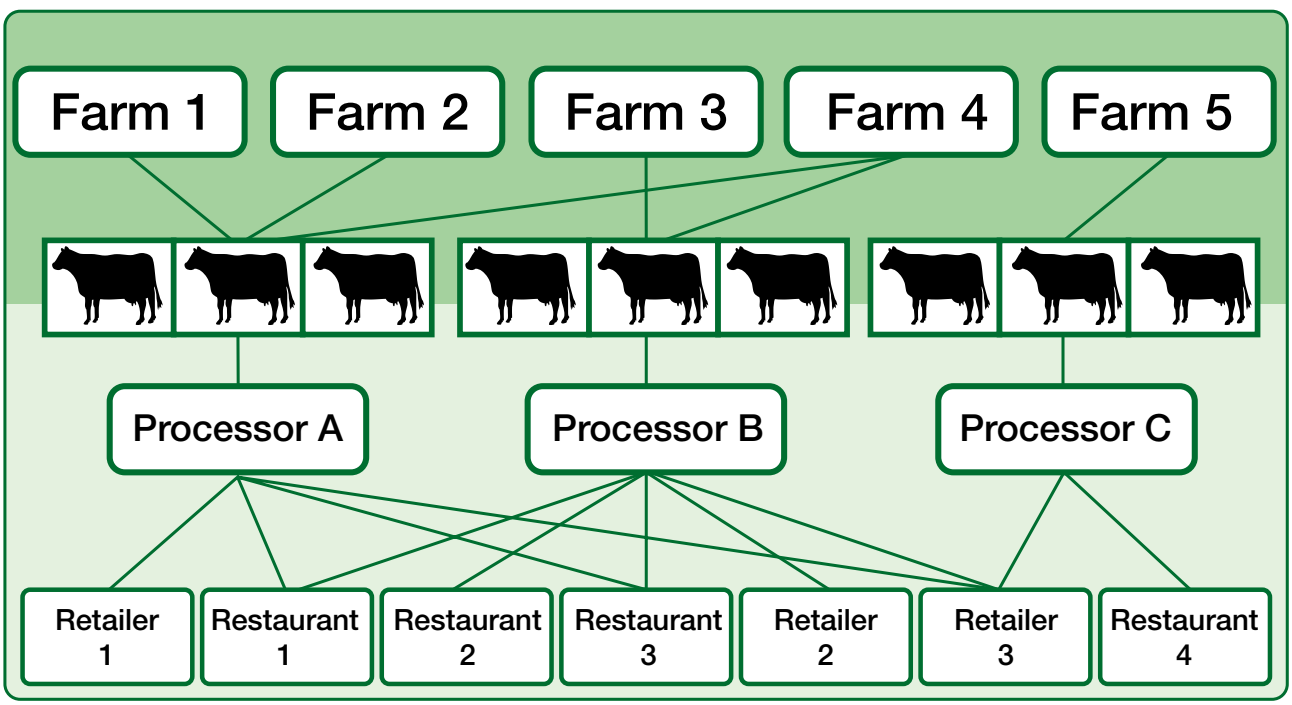


Figure 8. A simplified example of a beef supply chain (adapted from *Shackell*, 2008)

example of traceability "from farm to consumer" in the beef SC to further illustrate direct and indirect tracking of a sold product.

Figure 8 shows that fast tracking from farm to consumer can be both direct and indirect. In this diagram, the meat consumed in restaurants 1 and 3 is supplied from two processing slaughterhouses, A and B, where their meat is also supplied from cattle raised in four farms 1, 2, 3, and 4, one of which (farm 4) supplies meat to two processing slaughterhouses. Although retailers 1 and 2 provide meat from only one slaughterhouse (A and B, respectively), each of these slaughterhouses (A and B) is supplied by more than one farm, and farm 4 is common to both slaughterhouses. When there is a need for direct authentication of the sold meat, only the meat sold in Restaurant 4 can be traced to a single farm (Farm 5) among the four restaurants and three retailers. This is because Restaurant 4 purchases only from one slaughterhouse (C), and its meat came from only one livestock farm (5).

Given this example, adapted from *Shackell* (2008), it is understandable that there are many barriers to the direct traceability of meat in most cases, so the authenticity and origin of the consumed meat mostly depends on indirect tracking. Therefore, the design of traceable meat systems with indirect tracking capability, which also increases costs with the increasing the accuracy of their output information, is largely justified economically from the viewpoint of human health and is accepted by governments and consumers (*Jansen et al*., 2016; *Cheraghi Saray and Rafat*, 2016).

5.1.8 The role of regulatory and governmental authorities in meat traceability systems

Studies have shown that significant efforts have been made to draw meat traceability maps by different countries. China and the European Union have made the greatest efforts to address general consumer concerns about meat source traceability (*Jansen et al*., 2016; *Qian et al.*, 2020b). Since the consumer is the main motivation factor for designing global tracking systems, *Zhen et al.* (2019) carried out comprehensive studies in this regard and reported that some consumer behaviors toward food safety and risk factors were sometimes irrational. Hence, it can be suggested that consumers' tractability probably differs with the economic situation of each society (Thesmar and Stevens, 2019; Zhang et al., 2020). According to *Zhen et al.* (2019) and *Qian et al.* (2020b), traceability is a solution for consumer protection rather than a tool for the control of responsibilities. However, if consumer health is threatened by product(s), the producer will be able to troubleshoot via examining the various stages of the tracking system and make the necessary corrections. If the risk factors are of external origin or the corrections are beyond the capacity of the production unit, this is reported to the relevant authorities or institutions as soon as possible. However, retailers, wholesalers, and, in many cases, legislators insist on addressing tracking requests from the consumer perspective (*Sargeant et al*., 2007; *Wang et al*., 2018). As a result, some policies have been formulated in most countries such that the various stages of tracking systems are mainly implemented by consumer group representatives, private companies, and individual businesses, with governments ultimately making management decisions at the national and macro levels (*Salih*, 2017; *Wang et al*., 2018; *Galvez et al*., 2018; *Bougdira et al*., 2019; *Zhen et al.*, 2019). Therefore, there will be differences in the implementation of these systems in different countries, for which the main reasons are as follows: 1) the national livestock information system is unique for each country and is supported and implemented under the national laws of that country; 2) no two countries are exactly the same in terms of distance, nature, structure, and industry in the food SC, and; 3) different cultures in the agricultural industry of each country, and even each region, have a significant impact on the acceptability of traceability maps (*Cheraghi Saray and Hosseinkhani*, 2013; *Cheraghi Saray and Rafat*, 2016).

5.1.9 Costs and benefits of traceability systems

The main costs of companies or institutions that initially launch and design tracking systems are: 1) hardware costs, such as providing computers and scanners; 2) software costs, including purchasing applications tailored to each tracking system, such as the Abaserve; 3) costs of obtaining relevant licensing from national and international organizations; 4) costs of designing labels suitable for the type of meat sources; 5) costs of staff salaries, and; 6) costs of designing and maintaining central databases (*Vander-Merwe and Kirsten*, 2015). The main advantages of designing traceability systems are: 1) increased trust between meat producers (livestock owners and farmers), slaughterhouses, and consumers of meat products; 2) better control regarding the origin of meat through the use of electronic than paper-based documentation; 3) inventory control, online and accurate statistics, and limiting product theft; 4) improved control of illegal and

fraudulent cases; 5) correct identification of an incident problem at any stage from production to sale; 6) improved management and accounting units in relevant companies and institutions; 7) easy and accurate access to retail markets, and; 8) potentially increased health and safety of consumers (*Probst et al*., 2013; *Vander-Merwe and Kirsten*, 2015; *Galvez et al.*, 2018; *Zhang et al*., 2020).

6. Conclusion

This review has shown that for those involved in the meat SC, the implementation of modern electronics based on communication and information technology, such as electronic barcodes, DNA markers, RFID, GPS, EPCIS, and other biometric sensors, plays critical roles in monitoring and detecting problems and providing consumers with information to support their purchasing decisions. An important result of this review is the description of the implementation of different information systems and traceability in the meat product sector. Since the expected result of the current review was to explain the necessity, efficiency, and economic reasons for implementing tracking systems and provide guidance for future research, such studies are advised to examine consumer trends regarding meat SC traceability. Proper, full traceability would enable meat SC companies to limit their legal and financial burdens, would support production decisions, enhance consumer health and purchase decisions, and would create public confidence in meat chain security. Consequently, traceability in the meat SC can bring both commercial and regulatory benefits for any country.

Određivanje i primena alata za sledljivost u lancu snabdevanja mesom i proizvodima od mesa sa ciljem podizanja svesti potrošača i jačanja poverenja javnosti

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INFORMACIJE O RADU

Ključne reči:
Sledljivost
Izvori mesa
Proizvodi životinjskog porekla
Zdravlje životinja
Javno zdravlje

APSTRAKT

Zbog zabrinutosti za bezbednost uvezenih prehrambenih proizvoda i prevenciju zoonotskih bolesti vlade su se usmerile na dizajn sistema za praćenje. U ovoj studiji pregledana je i predstavljena potrebna infrastruktura, metode prikupljanja podataka, kao i zdravstvene koristi i komponente koje se postižu primenom sledljivosti na međunarodnom nivou. Pregled je pokazao da implementacija svakog elektronskog sistema za praćenje omogućava identifikaciju konzumiranog mesa od farme do trpeze. Međutim, sistemi za identifikaciju putem radio-frekvencije (RFID), DNK markeri i bežične senzorske mreže (WSN) označeni su kao najprikladnije i najtačnije metode za praćenje porekla konzumiranog mesa. Prema rezultatima istraživanja u ovom radu, regulatorna tela i donosioci odluka treba ozbiljno da obrate pažnju na ovo pitanje kako bi se sprečila pojava falsifikovanih proizvoda od mesa i očuvalo javno zdravlje.

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Original Scientific Paper

Possibility of extending shelf life of cevapcici

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ABSTRACT

The shelf life of cevapcici in which chemical additives were substituted with biopreservatives was examined. Bioprotective culture B-2 SafePro (Lactobacillus sakei) in freeze-dried form and the herb mixture, Oregano Plus, composed of oregano (Origanum vulgare L. ssp. Viridis) and savory (Satureja montana L.), were used. Three types of cevapcici were produced: with B-2 SafePro (control); with Oregano Plus, and; with both B-2 SafePro and Oregano Plus. pH, grilling weight loss, chemical composition, sensory characteristics, thibarbituric acid reductive substances (TBARS), and microbiological profile were investigated in all cevapcici treatments. The pH decreased in all treatments after 7 days' storage at 0–4 °C. Cevapcici with B-2 SafePro had a significantly ($P<0.05$) lower pH than the other two treatments after cold storage. A statistically significant negative correlation between pH and grilling weight loss of cevapcici was found ($P<0.01$). Consequently, the highest weight loss during grilling was found in cevapcici that contained B-2 SafePro. Products that contained both B-2 SafePro and Oregano Plus had the most acceptable sensory attributes three days after production. However, in cevapcici with B-2 SafePro (compared with the other two products), all sensory properties were significantly ($P<0.05$) better seven days after production. After frozen storage, significantly ($P<0.05$) lower TBA-numbers in the cevapcici with Oregano Plus indicate that this herb mixture has evident antioxidative effects. Products with B-2 SafePro had the highest total bacteria count, as a result of intensive growth and development the L. sakei. After 7 days' storage at 0–4 °C, the most expressive effect against Enterobacteriaceae was detected in cevapcici with Oregano Plus compared with the other two producs. Generally, it can be concluded that the addition of the oregano and savory mixture results in cevapcici that are microbiologically safe and have extended shelf life

1. Introduction

Consumer perception of meat products has changed in recent years, resulting in increased interest in healthier meat products (*Selani et al*., 2022). Consumer health care is oriented towards extending the shelf life and appropriate microbiological quality of meat and meat products. In response to this demand, there is a constant need to introduce new technologies in the food industry (*Jarmoluk et al.,* 2005).

Fresh meat products are typically sold at refrigerated temperatures (2–5 °C). However, various unwanted product changes, such as microbial growth and lipid oxidation, can occur during cooling, leading to reduced quality, meat spoilage, and financial losses (*Sallama & Samejima*, 2004).

Cevapcici are fast food products found traditionally in the countries of Southeast Europe. These meat products have been produced in the region since the Ottoman expansion over the Balkans. Today, they are considered a national dish of

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the Balkans, and are made from minced meat and various added spices. Cevapcici have a short shelf life of a maximum of 72 hours compared to meat in pieces. Storage of cevapcici, under aerobic conditions, enables growth of bacteria from the genus of *Pseudomonas* which causes changes in their texture, color, smell, and taste (*Gill*, 1986).

A recent trend in food production is to decrease synthetic additives, which have been vastly used because of the growing concern among consumers about their serious effects on human health (*Elzamzamy*, 2014). Consequently, the development and utilization of natural products with combined antioxidant and antibacterial activities in meat products could be essential and beneficial for extending their shelf life and reducing the risk of foodborne diseases (*Fernández-López et al*., 2004). Plant-derived compounds have been effective in reducing lipid oxidation in meat products (*Estévez et al.,* 2005). Many herbs, spices, and their extracts have been added to a variety of foods to improve their sensory characteristics and extend shelf life. Herbs belonging to the *Lamiaceae* family, primarily oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), and sage (*Salvia officinalis* L.), have been identified as possessing significant antioxidant properties (*Velasco & Williams*, 2011) attributable to three mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (*Castillo et al.,* 2013). Oregano is an aromatic perennial herb, with bioactive constituents, such as carvacrol and thymol, which possess several medicinal properties, namely antioxidant, antidiabetic, anti-inflammatory, antimicrobial, antiviral, antiparasitic, anti-neoplastic, and immune modulatory (*Alagawany et al*., 2020). *Origanum vulgare* L. ssp.Viridis is used to cure respiratory diseases (*Van Den Broucke & Lemli*, 1980), hypoglycemic disease (*Lemhardi et. al.,* 2004) and leukemia (*Goun et al*., 2002). *Satureja montana* L., commonly known as winter savory or mountain savory, also belongs to the *Lamiaceae* family and originates from the Mediterranean, but is widespread in Europe, Russia and Turkey. This highly aromatic herb has been traditionally used as a seasoning for food and an ingredient in teas for centuries (*Oliveira et al*., 2012). The high antimicrobial activity of savory can be attributed to major compounds, such as carvacrol, thymol, terpinen-4-ol and linalool (*Dorman & Deans,* 2000), and can be used to maintain meat quality, extend product shelf life, and prevent economic losses (*Yin & Cheng*, 2003).

Therefore, the purpose of the present investigation was to evaluate the possibility of extending the shelf life of cevapcici in which chemical additives were substituted with biopreservatives.

2. Materials and Methods

2.1 Production of cevapcici

Cevapcici were produced under industrial conditions, and the composition of the three products is given in Table 1. A mixture of reductive agents consisting of E300 (ascorbic acid), E316 (sodium erythorbate) and E330 (citric acid) was used. Bioprotective culture B-2 SafePro *(Lactobacillus sakei*) (Chr. Hansen, Denmark) in freeze-dried form and the herb mixture Oregano Plus (Alkaloid AD Skopje, R. N. Macedonia), composed of oregano (*Origanum vulgare* L.) and savory (*Satureja montana*), were used. In three replications, three treatments of cevapcici were produced: R\L with B-2 SafePro, R\O with Oregano Plus, and R\O\L with combined use of B-2 SafePro and Oregano Plus.

After production, the cevapcici were wrapped in cling film, placed in a cardboard box and stored at 0–4 °C .

2.2 Analytical methods

The pH of cevapcici was measured by a pH meter (pH-540 GLP, WTW, Germany).

The difference in weight (shrinkage) of cevapcici before and after grilling expressed as a percentage of cevapcici weight before grilling was defined as the weight loss of thermally processed cevapcici.

The degree of lipid oxidation in cevapcici was determined by TBARS test according to the method of Tarladgis et al. (1960), modified by Shahidi et al. (1983 and 1987). TBARS number was determined in grilled cevapcici (stored overnight in a refrigerator at a temperature of 0–4 °C).

The investigation of sensory characteristics was performed according to the score-pointing method (*Radovanović & Popov-Raljić*, 2000). The external appearance and color were assessed in fresh (raw, thermally unprocessed products), while the other sensory characteristics were assessed in grilled cevapcici.

Microbiological analyses of cevapcici were made according to the following methods: total number of aerobic bacteria ISO 4833:2013, *Listeria monocitogenes* ISO 11290-1, *Salmonella sp.* ISO 6579-1, *Campylobacter* ISO 10272-1, *Yersinia enterocolitica*

Table 1. Composition of cevapcici (%)

Ingredients	Treatments[1]		
	R\L	R\O	R\O\L
Beef (70% fresh and 30% frozen)	65	65	65
Chicken gut (frozen)	13	13	13
Textured soy	10	10	10
Fresh onion	8	8	8
Soy flour	4	4	4
Salt	1.8	1.8	1.8
Polyphosphates	0.3	0.3	0.3
Soy isolate	1	1	1
Ground black pepper	0.45	0.45	0.45
B-2 SafePro™	0.25	-	0.25
Oregano Plus	-	0.2	0.2

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro; R\O produced with Oregano Plus and; R\O\L produced with combined use of B-2 SafePro and Oregano Plus

ISO 10273, *E. coli* O157:H7 ISO 16654 and *Enterobacteriaceae spp* ISO 21528:2017 (*Official Gazette of R. Macedonia*, 2018 and 2022).

Statistical evaluation of data obtained from the research was performed by variance analysis (ANOVA), using the statistical package SPSS.

3. Results and Discussion

The pH measurements of cevapcici at three and seven days after production are presented in Table 2. There was a pH decrease in all treatments. R\L cevapcici had significantly ($P < 0.05$) lower pH at three or seven days after production than the other two treatments. Between R\O and R\O\L products, the differences in pH were statistically insignificant. The presence of the lactic acid bacterium *L. sakei* in R\L products caused significantly more acidification compared to the other cevapcici types. It is assumed that the higher pH in R\O\L products was due to the inhibitory effect of the phenolic components of the herbs on the lactic acid bacteria.

The pH values obtained in the present research were in accordance with the literature data. According to *Jelle* (1991), in B-2 SafePro products, carbohydrates are converted to lactic acid, resulting in a significant decrease in pH. *Jałosińska & Wilczak* (2009) reported that the addition of various plant extracts at 0.2% to meatball products resulted in little change in product acidity. On the 8th day of storage, pH reached 6.05 in meatballs with rosemary and 6.08 in meatballs with lovage (*Jałosińska & Wilczak*, 2009). According

Table 2. pH of cevapcici, three and seven days after production

Time (days)	Treatments[1]								
	R\L			R\O			R\O\L		
	M	S	C	M	S	C	M	S	C
pH_3	6.05[a]	0.10	0.01	6.13[b]	0.03	0.01	6.12[b]	0.30	0.01
pH_7	5.78[a]	0.10	0.02	6.02[b]	0.11	0.02	6.02[b]	0.08	0.01

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro, R\O produced with Oregano Plus and, R\O\L produced with combined use of B-2 SafePro and Oregano Plus; [a, b] Means in rows with different superscripts are significantly different ($P < 0.05$); M = mean, S = standard deviation, C = coefficient of variation

to *Verplaetse* (1994) and *Molly et al.* (1997), acidification of meat products, which occurs as a result of the proliferation of lactic acid bacteria, has many positive effects: reduction of the pH; ensuring hygienic stability; obtaining a characteristic sour taste; coagulation of proteins in meat; reduction of water holding capacity, and; development of a desirable red colour by favoring the reaction between nitrogen monoxide and myoglobin.

A statistically significant negative correlation ($R = -0.357$) between the cevapcici's pH and grilling weight loss was found ($P < 0.01$). Consequently, the highest weight loss during grilling was found in R\L cevapcici (Table 3). They were significantly ($P < 0.05$) different from the other two groups of cevapcici produced. The results obtained correspond to those of Kraft (1992), according to which weight loss during heat treatment increases with decreasing pH value.

From Table 4, it can be seen that TBARS values of cevapcici were low (< 0.60) seven days after storage (0–4 °C), but after 90 days' frozen storage (−18 °C) were higher (> 0.80) in R\L and R\O\L treatments. Significantly ($P < 0.05$) lower TBA numbers in R\O products than in the other two products after the frozen storage indicate that the herb mixture Oregano Plus has an evident antioxidative effect. In other studies, reduction of meat oxidation during refrigeration was obtained by adding oregano and sage essential oils to beef meat (*Fasseas et al.,* 2008) or even spraying a rosemary and vitamin C solution onto the surface (*Djenane et al.,* 2003). In addition, incorporation of oregano, rosemary, and sage essential oils into meats can delay lipid oxidation during refrigerated and frozen storage (*Velasco & Williams*, 2011).

Table 5 presents the results of the sensory analysis of cevapcici three and seven days after their production. Besides the average grades given by panelists, adjusted averages are presented. It is known that ordinary average grades are not a real indicator of the general quality of the product, because the characteristics evaluated are not equal in importance for the total quality, so correction (C) using appropriate coefficients of importance (CI) was required. Taste and smell , without doubt, have a significant share in the overall assessment.

It is noticeable that three days after production, R\O\L products had the most acceptable sensory attributes among the cevapcici groups, except for the external appearance which was most acceptable in R\L cevapcici. The pointed mean value and the percentage of maximum possible quality were higher in R\O\L cevapcici than in the other cevapcici

Table 3. Grilling weight loss of cevapcici, three and seven days after production (%)

Time (days)	Treatments[1]								
	R\L			R\O			R\O\L		
	M	S	C	M	S	C	M	S	C
3 days after production	15.20[a]	2.49	0.16	13.16[b]	2.91	0.22	14.91[b]	3.10	0.21
7 days after production	17.10[a]	2.80	0.17	15.18[b]	2.96	0.19	15.35[b]	1.83	0.12

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro, R\O produced with Oregano Plus and, R\O\L produced with combined use of B-2 SafePro and Oregano Plus; [a, b] Means in rows with different superscripts are significantly different ($P < 0.05$); M = mean, S = standard deviation, C = coefficient of variation

Table 4. Average TBARS (mgMDA/kg product) values of cevapcici, 7 and 90 days after production

Time (days)	Treatments[1]								
	R\L			R\O			R\O\L		
	M	S	C	M	S	C	M	S	C
7 days after production	0.52[a]	0.23	0.44	0.41[a]	0.18	0.44	0.47[a]	0.18	0.38
90 days after production	0.87[a]	0.09	0.10	0.51[b]	0.01	0.02	0.96[a]	0.04	0.04

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro, R\O produced with Oregano Plus and, R\O\L produced with combined use of B-2 SafePro and Oregano Plus; [a, b] Means in rows with different superscripts are significantly different ($P < 0.05$); M = mean; S = standard deviation; C = coefficient of variation

Table 5. Sensory evaluation of cevapcici, three and seven days after production

Sensory characteristics	CI	Treatments[1]											
		3 days after production						7 days after production					
		R\L		R\O		R\O\L		R\L		R\O		R\O\L	
		S	C	S	C	S	C	S	C	S	C	S	C
External appearance	**1**	4.0	4.0	3.8	3.8	3.9	3.9	4.5	4.5	3.5	3.5	4.0	4.0
Cross section appearance	**4**	4.2	16.8	4.1	16.4	4.4	17.6	4.3	17.2	3.9	15.6	3.8	15.2
Texture	**3**	4.2	12.6	3.9	11.7	4.5	13.5	4.6	13.8	4.3	12.9	3.9	11.7
Colour	**3**	3.9	11.7	3.7	11.1	4.1	12.3	4.5	13.5	3.8	11.4	4.0	12
Smell	**4**	4.3	17.2	4.1	16.4	4.4	17.6	4.6	18.4	4.1	16.4	3.7	14.8
Taste	**5**	4.1	20.5	4.2	21	4.3	21.5	4.5	22.5	4.3	21.5	3.7	18.5
Total CI	**20**												
Pointed mean value			4.14		4.02		4.32		4.49		4.06		3.81
% of maximum possible quality			82.8		80.4		86.4		89.9		81.3		76.2

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro, R\O produced with Oregano Plus and, R\O\L produced with combined use of B-2 SafePro and Oregano Plus; CI = Coefficient of importance; S = Score; C = Corrected score

groups at three days post-production. However, at seven days after production, all sensory properties were better in R\L cevapcici than in the other two cevapcici groups.

The results presented in Table 6 indicate that three days after production, the R\O\L and R\L cevapcici had significantly ($P < 0.05$) better texture compared to R\O products. For the other sensory attributes, the differences between treatments were statistically insignificant.

Seven days after production (Table 7), a significant ($P<0.05$) difference between the groups was recorded in all the tested sensory properties, except for the cross-section appearance. Cevapcici with B-2 SafePro had higher scores of all investigated sensory characteristics than did the other two cevapcici groups. The texture, smell, and taste were also highly rated for the cevapcici with a mixture of oregano and savory, but their external appearance and colour were unsatisfactory.

Table 6. Comparative overview of basic statistical parameters of sensory properties of cevapcici, three days after production

Sensory characteristics	Treatments[1]								
	R\L			R\O			R\O\L		
	M	S	C	M	S	C	M	S	C
External appearance	4.00[a]	0.97	0.24	3.78[a]	0.95	0.25	3.95[a]	0.91	0.23
Cross section appearance	4.22[a]	0.75	0.17	4.08[a]	0.72	0.18	4.38[a]	0.64	0.15
Texture	4.22[a]	0.85	0.2	3.92[b]	0.89	0.23	4.49[a]	0.65	0.14
Colour	3.95[a]	0.91	0.23	3.7[a]	0.88	0.24	4.11[a]	0.13	0.03
Smell	4.27[a]	0.73	0.17	4.08[a]	1.01	0.25	4.38[a]	0.72	0.16
Taste	4.11[a]	0.73	0.18	4.16[a]	0.9	0.22	4.32[a]	0.67	0.15

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro, R\O produced with Oregano Plus and, R\O\L produced with combined use of B-2 SafePro and Oregano Plus; [a, b] Means in rows with different superscripts are significantly different ($P < 0.05$); M = mean; S = standard deviation; C = coefficient of variation

Table 7. Comparative overview of basic statistical parameters of sensory properties of cevapcici, seven days after production

Sensory characteristics	Treatments								
	R\L			R\O			R\O\L		
	M	S	C	M	S	C	M	S	C
External appearance	4.55[a]	0.56	0.12	3.52[b]	0.87	0.25	4.00[b]	0.71	0.18
Cross section appearance	4.30[a]	0.69	0.16	3.94[a]	0.86	0.22	3.79[a]	0.92	0.24
Texture	4.61[a]	0.56	0.12	4.27[ab]	0.72	0.17	3.91[b]	0.92	0.24
Colour	4.48[a]	0.67	0.15	3.79[b]	0.96	0.25	4.03[ab]	0.73	0.18
Smell	4.58[a]	0.56	0.12	4.06[ab]	1.03	0.25	3.70[b]	1.07	0.29
Taste	4.52[a]	0.75	0.16	4.33[a]	0.78	0.18	3.70[b]	0.18	0.05

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro, R\O produced with Oregano Plus and, R\O\L produced with combined use of B-2 SafePro and Oregano Plus; [a, b] Means in rows with different superscripts are significantly different ($P < 0.05$); M = mean, S = standard deviation; C = coefficient of variation

The addition of bioprotective cultures in shaped minced meat products creates a chain of reactions that cause desirable changes in the products' sensory characteristics (*Verplaetse, 1994*). A stable colour and desired texture and flavour were obtained in hamburgers with a bioprotective culture (*Erkes,* 2011). *Jaspal et al.* (2021) found that oregano oil at a level of more than 0.2% (w/w) negatively affects the colour and sensory properties of chicken meat. The application of 1% oregano essential oil increased lightness and hue and decreased redness, whereas 0.5% of the oil did not affect pork color (*Zduńczyk et al., 2023*).

Efenberger-Szmechtyk et al. (2021) pointed out that herbs and spices used as additives in food products to enhance their aroma and taste can also be good solutions for preservation and extension of the products' shelf life. This is due to polyphenols and other bioactive compounds in herbs that have antioxidant and antimicrobial characteristics.

Microbiological analyses of the tested cevapcici (Table 8) showed that pathogenic bacteria were not detected in any treatment. The total number of aerobic mesophilic bacteria at one and seven days after production was highest in R/L products (compared

Table 8. Presence of pathogenic bacteria, and the number of Enterobacteriaceae and total aerobic mesophilic bacteria, one and seven days after production

Bacteria	Treatments[1]					
	R\L		R\O		R\O\L	
	1 day	7 days	1 day	7 days	1 day	7 days
Campylobacter	-	-	-	-	-	-
Yersinia enterocolitica	-	-	-	-	-	-
Salmonella spp.	-	-	-	-	-	-
Listeria monocytogenes	-	-	-	-	-	-
E. coli O157:H7	-	-	-	-	-	-
Enterobacteriaceae spp.	4.50 log cfu/g	4.47 log cfu/g	4.36 log cfu/g	4.31 log cfu/g	4.30 log cfu/g	4.81 log cfu/g
Total aerobic mesophilic bacteria	5.30 log cfu/g	5.50 log cfu/g	4.84 log cfu/g	5.20 log cfu/g	4.30 log cfu/g	4.30 log cfu/g

Legend: [1] Cevapcici treatments: R\L produced with B-2 SafePro, R\O produced with Oregano Plus and; R\O\L produced with combined use of B-2 SafePro and Oregano Plus – Not detected

with the other two cevapcici groups) as a result of the *L. sakei* colony intensive growth and development. The most expressive anticoliform effect was detected in R\O products compared with the other two cevapcici products.

The antimicrobial activity of herbs and spices is a result of the interaction between specific biochemical components in the herbs/spices and the metabolic mechanisms inside the bacteria cells. Therefore, these biochemical components need to enter inside the cell to influence its function (*Vergara et al.,* 2020). The present results were in accordance with those found by *Zhou et al*. (2023), who studied the effects of natural plant extracts on meat product quality. According to those authors, reduction of the number of total aerobic mesophilic bacteria is probably the result of reduced pH caused by the activity of bioprotective cultures and the antibacterial effect of the spice mixture. According to *Burt* (2004) oregano has a suppressive effect on the growth and development of *Listeria monocytogenes, Salmonella* spp*., E. coli* O157:H7*, Bacillus cereus* and *Staphylococcus aureus* in meat products. Oregano essential oil (0.5% and 1%) could delay the growth of microorganisms and decrease the final counts of the spoilage microorganisms (*Skandamis & Nychas, 2001*).

4. Conclusion

Generally, it can be concluded that the addition of a mixture of oregano and savory results in products that are microbiologically safe and have an extended shelf life. A negative correlation (P<0.01) was found between pH and cooking loss that occurred on grilling the cevapcici. This means that decreasing the pH significantly (P<0.01) increases the cooking loss during cevapcici grilling. Lipid oxidation is almost completely prevented in products with Oregano Plus. Among the treatments, 90 days after production, a significantly (P<0.05) higher TBK number was recorded in cevapcici with Oregano Plus. Seven days after production, sensory evaluation showed products with the B-2 SafePro bioprotective culture were the best overall among the three cevapcici groups. The presence of pathogenic bacteria was not detected in any group of cevapcici. The total number of aerobic mesophilic bacteria is highest in products with bioprotective culture. This is due to the intensive growth and development of the lactic acid bacterium, *L. sakei*. In order to reduce the growth of *Enterobacteriaceae* in cevapcici during cold storage, the mixture of oregano and savory could be effective.

Mogućnost produženja roka trajanja ćevapčića

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INFORMACIJE O RADU

Ključne reči:
Ćevapčići
Origano
Vrijesak
Proizvodi od mesa
Rok trajanja

APSTRAKT

Ispitan je rok trajanja ćevapčića u kojima su hemijski aditivi zamenjeni biokonzervansima. Korišćena je biozaštitna kultura B-2 SafePro (Lactobacillus sakei) u liofilizovanom obliku i biljna smeša Origano Plus, sastavljena od origana (Origanum vulgare L. ssp. Viridis) i vrijeska (Satureja montana L.). Proizvedene su tri vrste ćevapčića: sa B-2 SafePro (kontrola); sa Origano Plus; i sa B-2 SafePro i Origano Plus. U svim tretmanima ćevapčića ispitivani su pH, gubitak mase na roštilju, hemijski sastav, senzorne karakteristike, reaktivne supstance tiobarbiturnekiseline (TBARS) i mikrobiološki profil. Vrednost pH se smanjila u svim tretmanima nakon 7 dana skladištenja na 0–4 °C. Ćevapčići sa B-2 SafePro su imali značajno (P<0,05) niži pH u odnosu na druga dva tretmana nakon skladištenja na hladnom. Utvrđena je statistički značajna negativna korelacija između pH vrednosti i gubitka mase ćevapčića na roštilju (P<0,01). Shodno tome, najveći gubitak mase tokom pečenja je zabeležen kod ćevapčića koji su sadržali B-2 SafePro. Proizvodi koji su sadržali i B-2 SafePro i Origano Plus imali su najprihvatljivije senzorne atribute tri dana nakon proizvodnje. Međutim, kod ćevapčića sa B-2 SafePro (u poređenju sa druga dva proizvoda) sve senzorne karakteristike su bile značajno bolje (P<0,05) sedam dana nakon proizvodnje. Nakon zamrznutog skladištenja, značajno (P<0,05) niži TBA-brojevi u ćevapčićima sa Origano Plusom ukazuju na to da ova biljna smeša ima evidentno antioksidativno dejstvo. Proizvodi sa B-2 SafePro su imali najveći ukupan broj bakterija, kao rezultat intenzivnog rasta i razvoja L. sakei.

Posle 7 dana skladištenja na 0–4 , najizrazitiji efekat protiv Enterobacteriaceae je otkriven kod ćevapčića sa Origano Plus u poređenju sa druga dva proizvoda. Uopšteno gledano, može se zaključiti da se dodavanjem mešavine origana i vrijeska dobijaju ćevapčići koji su mikrobiološki bezbedni i imaju produženi rok trajanja.

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Original scientific paper

Validation of pasteurization of finely chopped, cooked sausages with a small diameter

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Thermocouple

ABSTRACT

Pasteurization is a physical food preservation technique that effectively destroys microorganisms and inactivates tissue enzymes by applying moderate temperatures below 100°C. The safety and shelf-life of finely ground sausages, which are packaged in polyamide casings diameter 50 mm, weighing 220 grams, and produced by a food business operator, are ensured through pasteurization which lasted a total of 47 minutes. The standard pasteurization was performed in chamber at 80°C, in a saturated steam environment. Following this, the sausages underwent a cooling phase lasting 25 minutes in the same chamber immediately after the termination of pasteurization. During the pasteurization at 80°C and the cooling thereafter, pasteurization values (Pv) were ascertained in the thermal center of the sausages (thermocouple channels 2, 4, 5, 7, 8, 10, and 11), and ranged from 61.45 min (channel 10) to 81.07 min (channel 8). By achieving these Pv values, the temperature of 74°C in the thermal center of the cooked sausages was validated as adequate for ensuring the safety of the sausage product under the already-defined conditions of the cold chain storage.

1. Introduction

Thermal processing remains a primary technology for ensuring the safety of some foods. For cooked sausages, pasteurization as a traditional physical conservation method is generally used to kill microorganisms through heat introduction into the meat product structure that reaches the thermal center (*Basumatary et al.*, 2020). Quality and safety are paramount throughout the entire meat production and processing cycle. Meat and meat product processing focuses on enhancing quality, achieving desired sensory characteristics, improving digestibility, and prolonging shelf life (*Onopiuk et al.,* 2021). Pasteurization involves a gentle heat treatment applied to food, usually at temperatures below 100°C, designed to eliminate the vegetative cells of both pathogenic and most non-pathogenic microorganisms (*Benattouche et al.,* 2020). In the food industry, thermal pasteurization employs a range of techniques to guarantee microbiological safety (*Kamilla et al.*, 2024). For sausage products with a small diameter, safety is ensured by verifying the required temperature at the thermal center of the product, rather than by checking pressure-velocity values. To maintain the safety and quality of pasteurized, cooked sausages throughout their defined shelf life, it is essential that they are kept under specified cold chain conditions (between 0° and 4°C) during storage, transportation, and distribution **(***Raseta et al*., 2021).

Thermal processing can alter the flavor, taste, color, and nutritional values of the product. As a result, both the food industry and consumers are interested in developing new techniques

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that preserve the taste, colour and nutritional value of products, and that are also more energy-efficient than standard thermal methods (*Lalabadi et al*., 2023).

However, the growing consumer demand for convenient, easy-to-prepare foods that retain their nutritional value is shaping the current food market. Nowadays, there is growing demand for convenient food products that are minimally processed, high-quality, contain fewer additives, and have an extended shelf life. Thermal processing is utilized to reduce health risks from harmful microorganisms in low-acid foods and to prolong the shelf life for several days or weeks by eliminating spoilage microorganisms and/or inactivating enzymes. Special attention is devoted to the elimination of pathogenic microorganisms such as *Salmonella* spp, *Listeria monocytogenes* and *Escherichia coli* (*Ziyaina et al.*, 2020, *Bermudez-Aguirre and Niemira B.*, 2022). Bacteria that cause product spoilage (*Enterococcus* spp.*, Lactobacillus* spp.*, Micrococcus* spp.) are more heat-resistant than pathogenic bacteria (*Salmonella* spp., *Staphylococcus* spp.), and therefore, *Enterococcus faecium* is used as a reference microorganism to assess the effectiveness of pasteurization, as it is more thermoresistant than pathogenic microorganisms (*Vuković I*., 2012). Proper pasteurization effectively removes *Salmonella* from sausage batter (*Silva F. and Gibbs P.*, 2012).

During the logarithmic microbial destruction, different points are found on the resulting curve, whose distance determines the decimal reduction time, i.e., the D value. This is the heating time in minutes required to reduce the initial number of bacteria in suspension by 1/10, so is also the time in minutes required for the curve to pass through one logarithmic cycle. The guidelines for pasteurization processes are determined by the specific target bacterium (*Enterococcus faecium*) and the necessary heat treatment to achieve a minimum five-log reduction of that microorganism. The pasteurization process necessary to destroy *Enterococcus faecium* according to the 5D concept should produce a pasteurization value (*Pv, further Pv*) of > 40 min (*Vuković I.*, 2012).

The aim of thermal pasteurization is to extend the shelf life of food by destroying pathogenic microorganisms and decreasing the overall microbial load compared to raw food products (*Daelman et al*, 2013). An inadequate heating process could lead to degradation of proteins, vitamins, and other vital nutritional elements (*Hernández-Hernández et al.*, 2019). Therefore, the pasteurization process in the production facility must be reviewed by the HACCP team to maintain a high level of process control and ensure product safety without unnecessary energy losses or losses in value of food and nutrients.

Pasteurized meat products are subjected to the preservative effects of temperatures below the boiling point of water during production, ensuring that a minimum temperature of 70°C is reached in the thermal center. Alternatively, if the thermal treatment process allows for a temperature of at least 65°C to be reached in the thermal center, it must be maintained for a time duration sufficient to achieve a pasteurization value (Pv) ≥ 40 (*Serbia*, 2023). In the meat industry in Serbia, canned, smoked, and cooked sausages and other meat products are pasteurized at 75–85°C, and in the thermal center of the product, at least 70°C must be achieved (*Vukovic I.*, 2012). Previous production practices in the meat industry indicate that the median chamber temperatures are usually not higher than 82°C during sausage pasteurization (*Oluški V.,* 1973).

For many years, industrial practices in thermal pasteurization have relied on heat treatments deemed “safe harbors.” A safe harbor process is one that manufacturers can implement without requiring detailed information about the product or potential contamination risks.

To reduce costs and perhaps produce more desirable sausage products, these safe harbor treatments can be reduced, but any novel pasteurization treatment must be validated. Validation provides evidence that food hygiene control measures achieve effective and continuous management of food hazards at an appropriate level (*Serbia*, 2011). As *Codex alimentarius* stated, validation of control measures is needed to obtain evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome (*Codex alimentarius*, 2022). For the validation of pasteurization, it is crucial to position the control probe correctly at the thermal center of the product within the heating chamber. The personnel responsible must possess the necessary skills to consistently repeat this procedure during each pasteurization cycle to ensure accurate results. When commercializing an optimized pasteurization process, conducting shelf life studies on the optimized product is also essential. Additionally, maintaining the specified cold chain after production and during retail is critical to preserve product quality.

Sausage is made from ground meat or a mixture of different meats, combined with seasonings and spices, and then encased in a casing or container. According to current national legislation (*Serbia*, 2023), cooked sausages are defined as meat products made from meat, fatty tissue, connective tissue, offal, blood products, and additives. The filling can include meat dough, and these products are filled into casings or moulds before undergoing heat treatment at pasteurization temperatures, which may or may not include smoking. Various additives, such as salt, brining salts, water, spices, spice extracts, sugars, and flavourings, can be incorporated into the production of cooked sausages, including smoke and natural aromas. Finely chopped cooked sausages are produced by stuffing fillings into natural or artificial casings. The sausages are firm and juicy to the extent that they do not release water, with a pleasant characteristic flavor that is complemented by the aroma of smoke and spices. The sausage casing should be well filled with the filling, without damage, deformations, or folds (*Vukovic I*, 2012).

The objective of this work was to validate a pasteurization heat treatment of 74°C in the thermal centre of a commercial cooked sausage product, to determine whether the sausages are appropriately pasteurized.

Figure 1. Schroter chamber with thermocouple probes observed from the side in the thermal processing room.

2. Materials and Methods

The food business operator wanted to validate using a temperature of 74°C in the thermal centre of the sausages as part of its implemented food safety assurance system that involved hazard analysis and critical control points (HACCP). The validation of thermal treatment was conducted during the pasteurization of finely ground cooked sausages, 220 g in weight, stuffed into polyamide casings with a diameter of 50 mm. During the filling process, great care was taken to ensure that the casing was filled properly with the stuffing, avoiding any deformations or creases.

Measurements were obtained using the thermal validation system Ellab (E-Val Pro, serial number 411982, validated software — US FDA, 21 CFR part 11, GMP, ver. 4.6.1.0), and the technical report was prepared using the Ellab ValSuite software, version 5.2.015. Thermoelements with compensating cables were utilized, and temperatures were recorded at one-minute intervals. During the regular sausage production process, probes were placed in the thermal centres of multiple sausages and in the chamber medium. Measurements were obtained during regular production in a Schroter-type chamber (Figure 1). Four carts of product were placed in the chamber, with a total weight of 1040 kg (Figure 2). All probes were placed in the middle of the cart viewed from above, while a side view of probe placement is shown in Figure 2.

A total of 11 probes were used (thermocouple channels: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12), to record temperature and *Pv* value every minute, with seven probes placed in the thermal centres of sausages (thermocouple channels 2, 4, 5, 7, 8, 10, and 11) (Figure 3), while four probes were positioned in the chamber medium (thermocouple channels 3, 6, 9, and 12). Pasteurization was performed in the chamber at 80°C, in a saturated steam environment.

The temperature monitoring process continued after the active phase of pasteurization ended and during the first cooling phase until the temperature in the thermal center of the sausages was < 55°C (*Raseta et al.*, 2021). After completing the pasteurization process, the sausages were sent to the circulating air cooling room for further cooling. As the heat was transferred through the cooked sausages by

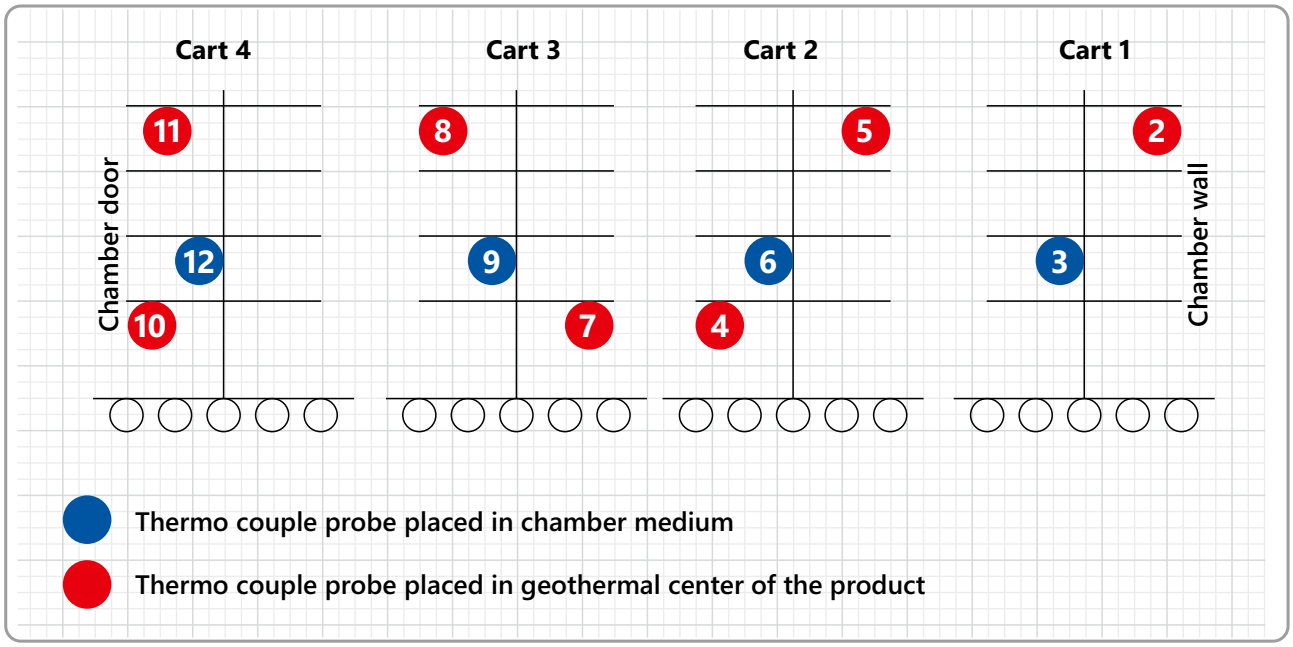

Figure 2. The positions of the probes installed in the Schroter chamber on carts, viewed from the side.

conduction, the temperature of the product should increase, both at the beginning of cooling and in the thermal center of the sausages, reaching about 74**°C.**

Statistical modeling using multiple linear regression (MLR) with prediction profiler was used to determine the time and temperature interval for achieving *Pv* > 40 min for all channels. JMP Statistical Discovery 10 (SAS Institute Inc. NC, USA https://www.jmp.com) was used for statistical analysis and presentation of results. MS Office 2016 Excel software was applied for exporting, sorting and preparing data for further analysis.

3. Results and Discussion

The thermal treatment process for finely ground cooked sausages (220 g in weight) packed in a polyamide casing, conducted in the Schroter chamber, lasted a total of 47 minutes, followed by a cooling phase of 25 minutes. The temperature of the filled sausage stuffing in the polyamide casing, prior to the start of thermal treatment, ranged from 16.71°C (channel 5) to 20.73°C (channel 7). At all verification points in the chamber, suitable *Pv* values of > 40 min were achieved in the thermal centres of the sausages (channels 2, 4, 5, 7, 8, 10, and 11), with values ranging from 61.45 (channel 10) to 81.07 (channel 8). The temperatures recorded in the sausages thermal centres during pasteurization, along with their corresponding *Pv* values, are presented in Table 1.

Temperature in the sausages' thermal centres and pasteurization values (*Pv*) achieved during pasteurization at 74°C.

The lowest *Pv* value in the red zone (Table 1, 40 min > *Pv* < 80 min) was recorded by channel 11 (41.78), while the highest was detected by channel 5 (79.29 min).

Theoretical modeling and predictive model for further improvement of the pasteurization process of the studied sausages.

Figure 3 was constructed from the data in Table 1. Prediction profiler indicated that an approximate temperature of 60**°C** is required to obtain *Pv* > 40 min after almost 47 minutes of heating. Also, as results of the prediction profiler demonstrated, any further temperature rise above 60°C would not have a further favourable effect.

3D scatter plots of *Pv* value vs time and temperature in sausage centers as recorded by thermocouple probe channels: a) channel 2; b) channel 4; c) channel 5; d) channel 7; e) channel 8; f) channel 10; g) channel 11. Colors of data points correspond to the colors in Table 1.

Figure 4 shows *Pv* vs temperature and time, as measured by thermocouple probes in the sausages. It is obvious that data in the red zone (from Table 1 and the red data points in Figure 4) covered the transitional range of *Pv* values for each channel. Upon analysing the results obtained, we can conclude that the heat treatment process for the studied sausages was consistent and uniform.

Table 1. Thermocouple measurements from probes 2, 4, 5, 7, 8, 10, and 11, placed in the geothermal center of the sausages over 73 minutes of process recording time

t (min)	T CH 2 (°C)	*Pv* CH 2	T CH 4 (°C)	*Pv* CH 4	T CH 5 (°C)	*Pv* CH 5	T CH 7 (°C)	*Pv* CH 7	T CH 8 (°C)	*Pv* CH 8	T CH 10 (°C)	*Pv* CH 10	T CH 11 (°C)	*Pv* CH 11
1	19.93	0	18	0	16.71	0	20.37	0	17.69	0	19.12	0	17.02	0
2	19.89	0	19.97	0	16.68	0	20.36	0	17.64	0	19.14	0	16.99	0
3	19.76	0	17.87	0	16.6	0	20.25	0	17.54	0	19.08	0	16.78	0
4	19.8	0	17.98	0	16.79	0	20.27	0	17.86	0	19.22	0	16.71	0
5	20.28	0	18.73	0	17.58	0	20.46	0	18.99	0	19.73	0	16.74	0
6	21.4	0	20.16	0	19.11	0	21.05	0	20.92	0	20.87	0	17.08	0
7	23.19	0	22.32	0	21.27	0	22.08	0	23.45	0	22.66	0	17.85	0
8	25.64	0	25	0	23.96	0	23.57	0	26.42	0	24.95	0	19.09	0
9	28.64	0	28.09	0	27.1	0	25.5	0	29.7	0	27.6	0	20.82	0
10	32.02	0	31.39	0	30.63	0	27.82	0	33.13	0	30.53	0	23.05	0
11	35.61	0	34.84	0	34.27	0	30.43	0	36.54	0	33.59	0	25.66	0
12	39.15	0	38.17	0	37.88	0	33.24	0	39.82	0	36.61	0	28.53	0
13	42.54	0	41.42	0	41.37	0	36.12	0	42.89	0	39.56	0	31.51	0
14	45.66	0	44.5	0	44.75	0	38.97	0	45.73	0	42.36	0	34.6	0
15	48.41	0	47.35	0	47.85	0	41.76	0	48.39	0	45.01	0	37.62	0
16	50.88	0.01	49.93	0.01	50.61	0.01	44.42	0	50.83	0.01	47.49	0	40.56	0
17	53.13	0.02	52.31	0.02	53.05	0.02	46.96	0	53.07	0.02	49.82	0.01	43.34	0
18	55.18	0.04	54.48	0.03	55.23	0.03	49.35	0.01	55.15	0.04	52.02	0.02	45.96	0
19	57.05	0.06	56.45	0.05	57.18	0.06	51.59	0.01	57.07	0.06	54.05	0.03	48.41	0
20	58.78	0.1	58.26	0.09	58.97	0.1	53.7	0.03	58.84	0.1	55.94	0.05	50.7	0.01
21	60.39	0.16	59.94	0.14	60.58	0.16	55.66	0.04	60.48	0.16	57.69	0.08	52.87	0.02
22	61.86	0.25	61.48	0.22	62.09	0.25	57.48	0.07	61.97	0.25	59.32	0.13	54.85	0.04
23	63.26	0.37	62.86	0.33	63.47	0.38	59.2	0.12	63.37	0.38	60.8	0.19	56.72	0.06
24	64.55	0.54	64.18	0.48	64.75	0.56	60.8	0.18	64.66	0.55	62.22	0.29	58.41	0.1
25	65.76	0.77	65.41	0.69	65.93	0.8	62.3	0.28	65.87	0.78	63.49	0.42	60.02	0.15
26	66.87	1.07	66.53	0.97	67.03	1.11	63.68	0.41	66.98	1.09	64.69	0.6	61.51	0.23
27	67.93	1.45	67.6	1.32	68.06	1.51	64.95	0.6	68.01	1.49	65.83	0.83	62.91	0.34
28	68.9	1.95	68.54	1.78	69.02	2.02	66.12	0.85	68.95	1.99	66.88	1.14	64.19	0.5
29	69.75	2.56	69.45	2.35	69.89	2.65	67.21	1.17	69.83	2.61	67.86	1.52	65.41	0.71
30	70.61	3.32	70.27	3.05	70.71	3.43	68.21	1.59	70.52	3.38	68.75	2.01	66.49	0.98
31	71.38	4.23	71.04	3.89	71.43	4.36	69.13	2.12	71.37	4.29	69.6	2.6	67.55	1.34
32	72.05	5.32	71.74	4.9	72.12	5.46	69.97	2.77	72.05	5.38	70.38	3.33	68.48	1.79
33	72.68	6.6	72.37	6.09	72.74	6.76	70.74	3.55	72.67	6.65	71.09	4.2	69.35	2.35
34	73.24	8.07	72.93	7.46	73.28	8.25	71.46	4.49	73.21	8.12	71.75	5.22	70.14	3.03
35	73.74	9.74	73.42	9.02	73.77	9.94	72.09	5.61	73.68	9.79	72.32	6.4	70.87	3.85
36	74.18	11.62	73.87	10.77	74.19	11.84	72.66	6.89	74.1	11.64	72.82	7.76	71.52	4.82
37	74.56	13.7	74.27	12.71	74.58	13.92	73.15	8.36	74.49	13.68	73.3	9.28	72.12	5.95
38	74.92	15.97	74.63	14.83	74.91	16.2	76.63	10	74.86	15.91	73.73	10.98	72.67	7.25
39	75.24	18.43	74.96	17.14	75.23	18.66	74.05	11.83	75.16	18.34	74.12	12.85	73.14	8.72

t (min)	T CH 2 (°C)	*Pv* CH 2	T CH 4 (°C)	*Pv* CH 4	T CH 5 (°C)	*Pv* CH 5	T CH 7 (°C)	*Pv* CH 7	T CH 8 (°C)	*Pv* CH 8	T CH 10 (°C)	*Pv* CH 10	T CH 11 (°C)	*Pv* CH 11
40	75.53	21.09	75.27	19.63	75.51	21.3	74.44	13.85	75.46	20.95	74.49	14.9	73.55	10.35
41	75.79	23.93	75.52	22.3	75.76	24.13	74.78	16.06	75.71	23.75	74.83	17.14	73.99	12.15
42	76.02	26.94	75.77	25.13	75.98	27.13	75.07	18.44	75.92	26.71	75.1	19.55	74.35	14.14
43	76.2	30.11	75.96	28.13	76.13	30.27	75.33	21	76.1	29.81	75.33	22.12	74.67	16.31
44	76.36	33.43	76.1	31.26	76.27	33.53	75.55	23.7	76.26	33.05	75.55	24.84	74.96	18.63
45	76.49	36.86	76.25	34.5	76.39	36.9	75.76	26.56	76.37	36.4	75.74	27.68	75.18	21.12
46	76.59	40.4	76.35	37.85	76.48	40.35	75.93	29.55	76.46	39.84	75.89	30.66	75.41	23.74
47	76.66	44.02	76.42	41.27	76.55	43.88	76.07	32.66	76.53	43.36	76.03	33.74	75.58	26.5
48	76.74	47.71	76.5	44.76	76.62	47.47	76.17	35.88	76.6	46.93	76.14	36.93	75.72	29.37
49	76.81	51.46	76.59	48.31	76.7	51.12	76.33	39.16	76.66	50.56	76.28	40.2	75.86	32.34
50	76.8	55.27	76.55	51.93	76.72	54.84	76.37	42.57	76.65	54.25	76.28	43.57	75.98	35.4
51	76.57	59.07	76.32	55.52	76.65	58.57	76.22	46.02	76.47	57.92	75.96	46.94	76.09	38.55
52	76.06	62.68	75.79	58.92	76.42	62.24	75.78	49.34	76.09	61.44	75.26	50.07	76.11	41.78
53	75.31	65.89	74.99	61.93	76.01	65.72	74.99	52.35	75.53	64.67	74.18	52.74	76.05	45.02
54	74.35	65.58	73.96	64.44	75.48	58.89	73.88	54.85	74.83	67.51	72.76	54.82	75.85	48.22
55	73.27	70.74	72.82	66.42	74.8	71.69	72.57	56.79	74.02	69.92	71.25	56.32	75.56	51.27
56	72.19	72.43	71.58	67.94	74.07	74.09	71.25	58.23	73.05	71.93	69.83	57.38	75.1	54.13
57	71.13	73.75	70.31	69.08	73.34	76.12	69.99	59.28	72.03	73.53	68.52	58.14	74.45	56.7
58	70.15	74.78	69.1	69.93	72.62	77.83	68.85	60.08	71.05	74.8	67.3	58.7	73.68	58.91
59	69.3	75.6	67.96	70.58	71.94	79.29	67.8	60.89	70.1	75.81	66.27	59.13	72.83	60.77
60	68.55	76.28	66.98	71.08	71.28	80.53	66.86	61.17	69.17	76.62	65.37	59.47	71.93	62.29
61	67.89	76.85	66.11	71.47	70.61	81.6	66.03	61.55	68.35	77.28	64.63	59.74	71	63.53
62	67.29	77.33	65.32	71.8	70.01	82.51	65.28	61.87	67.61	77.82	63.99	59.97	70.05	64.53
63	66.77	77.76	64.67	72.07	69.44	83.31	64.64	62.14	66.99	78.28	63.42	60.17	69.12	65.33
64	66.31	78.14	64.1	72.3	68.9	84	64.08	62.37	66.48	78.68	62.96	60.35	68.27	65.98
65	65.93	78.48	63.63	72.5	68.43	84.62	63.63	62.57	66.05	79.03	62.58	60.5	67.5	66.51
66	65.6	78.79	63.23	72.69	67.98	85.18	63.24	62.76	65.69	79.35	62.25	60.65	66.8	66.96
67	65.31	79.08	62.93	72.85	67.58	85.67	62.92	62.92	65.39	79.64	61.96	60.78	66.24	67.34
68	65.03	79.35	62.63	73.01	67.22	86.13	62.65	63.08	65.11	79.92	61.72	60.9	65.72	67.67
69	64.8	79.6	62.4	73.16	66.85	86.55	62.42	63.23	64.84	80.18	61.49	61.02	65.26	67.97
70	64.59	79.84	62.18	73.29	66.54	86.93	62.23	63.36	64.6	80.42	61.33	61.13	64.87	68.24
71	64.38	80.07	62	73.42	66.23	87.29	62.05	63.5	64.38	80.65	61.15	61.24	64.52	68.48
72	64.19	80.29	61.84	73.55	65.94	87.62	61.92	63.62	64.18	80.86	61	61.35	64.19	68.71
73	64.02	80.49	61.69	73.67	65.67	87.94	61.81	63.75	63.99	81.07	60.84	61.45	63.91	68.92

CH, thermocouple channel. For all channels, In the green zone, Pv values showed rapid gain with increasing temperature the Red zone represents the interval of 40 min < Pv < 80 min. In the blue zone, only slight or no further increases were recorded.

To effectively implement HACCP principles, it is crucial to utilize microbiological data gathered during the system's validation. Additionally, the HACCP system should be verified throughout the implementation process (*Konstantinos T et al.*, 2014).

It is necessary to compare the reported results with existing thermal profiles to determine the accuracy of the measuring instruments in the Schroter chamber. The P*v* values obtained in this study, i.e., 61.45 min – 81.07 min, were comparable to those found in other, similar research (*Raseta et al.*, 2021)

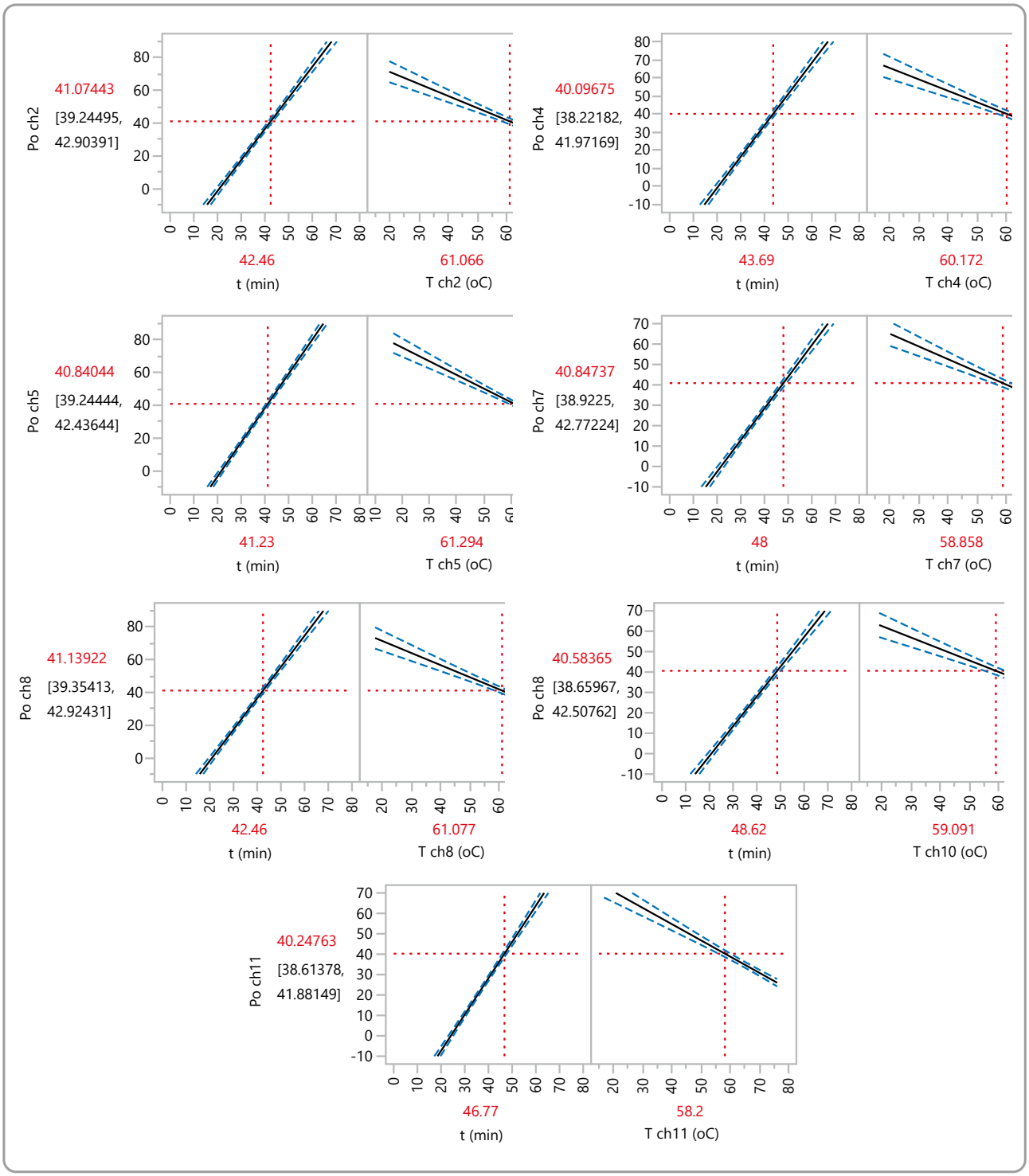

Figure 3. Theoretical modeling and predictive model for further improvement of the pasteurization process of the studied sausages

on optimized finely chopped cooked sauasges with a wider diameter (75–90 mm), wherein *Pv* values were 58.4 min to 97.64 min.

Theoretically, according to the statistical model applied in the current study, it would be possible for the food business operator to further optimize the pasteurization process, by maintaining the chamber temperature at 60°C, which would achieve $Pv > 40$ min after 50 minutes of heating. This lower-tempreature pasteurization regime would expose the sausages to significantly lower temperatures, which would positively affect the biological value, the presence of nutrients, and the degree of degradation of the additives used in the production of these sausages.

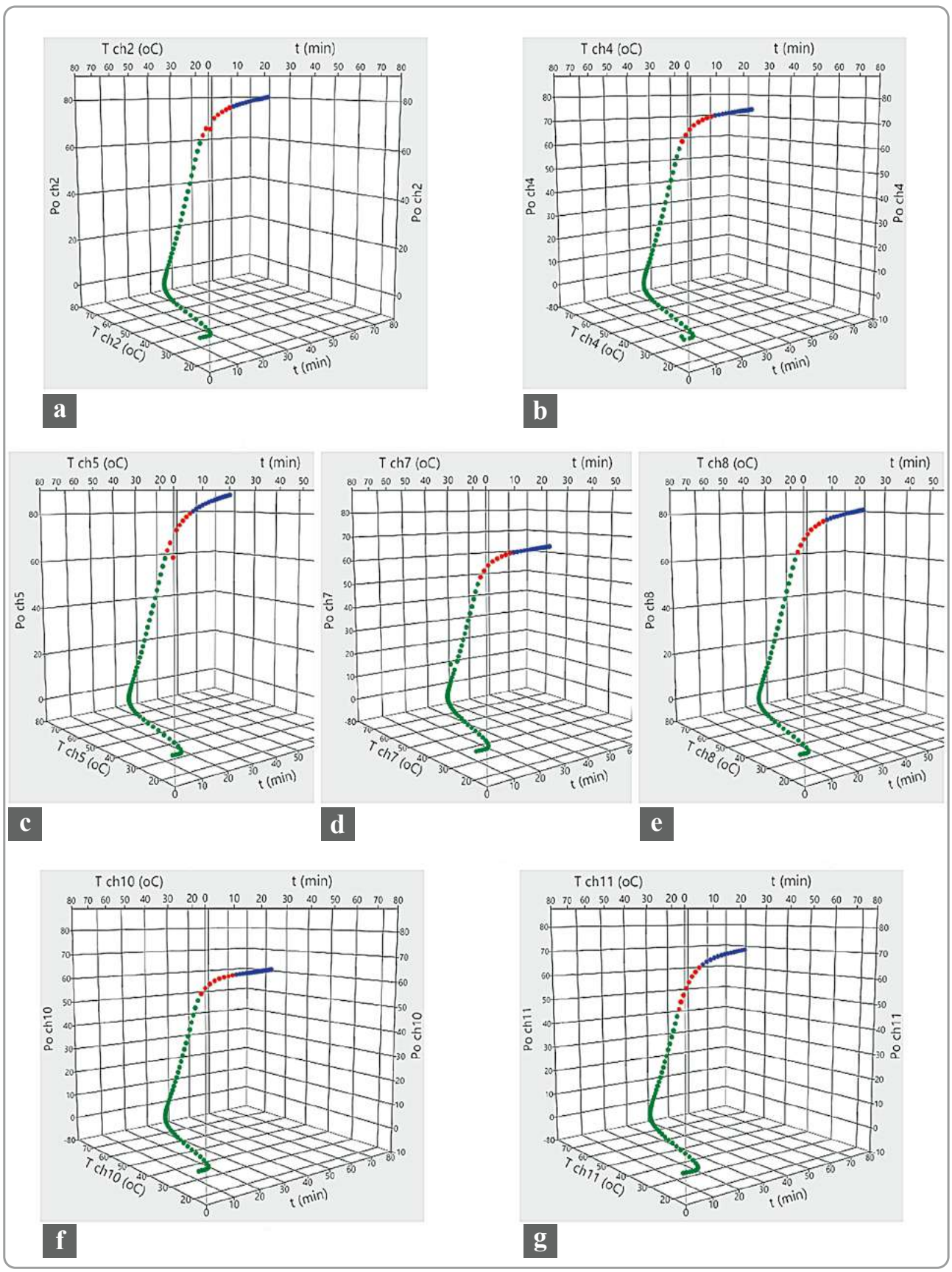

Figure 4. 3D scatter plots of pv value vs time and temperature in sausage centers as recorded by thermocouple probe channels: a) channel 2; b) channel 4; c) channel 5; d) channel 7; e) channel 8; f) channel 10; g) channel 11. Colors of data points correspond to the colors in Table 1

4. Conclusion

Thermal pasteurization is a crucial process in the meat industry for producing high-quality sausage products that have an extended shelf life if they are correctly stored at the specified refrigeration temperatures.

Validation of the pasteurization temperature of 74°C was performed by determining the *Pv* value achieved in the thermal centers of the studied sausages. The *Pv* values were within the range of 61.45 min – 81.07 min.

Theoretically for the studied sausages, the pasteurization process could be optimized. The suggested pasteurization regime suggested by the authors is 50 minutes of heating with the temperature maintained at 60°C, according to the applied MLR statistical model, which would achieve a *Pv* > 40 min. This suggested lower-tempreature pasteurization regime would expose the product to significantly lower temperatures than currently is the case, which would positively affect the biological value, the presence of nutrients, and the degree of degradation of the additives used in the production of these sausages.

The food business operator now has the opportunity to oversee the entire thermal processing process, with a good level of control over the entire process and ensuring the safety of their finely ground, cooked sausages in any zone of the pasteurization chamber. Nonetheless, validation of any change to the pasteurization regime is needed to produce documented evidence that provides a high degree of assurance that the specific process will consistently produce sausages that meet the business' predetermined specifications and quality attributes.

Validacija pasterizacije fino usitnjenih barenih kobasica užeg dijametra

Mladen Rašeta, Ivana Branković Lazić, Boris Mrdović, Nikola Betić, Becskei Zsolt, Jelena Jovanović i Radivoj Petronijević

INFORMACIJE O RADU

Ključne reči:
Pasterizacija
Validacija
Barene kobasice
Toplotna obrada
Termokapl

APSTRAKT

Pasterizacija je fizički metod konzervacije proizvoda od mesa, koja efikasno unišata vegetativne oblike mikroorganizama i inaktivira tkivne enzime, primenom temperature ispod 100°C. Bezbednost i održivost fino usitnjenih kobasica, pakovanih u poliamidni omotač dijametra 50 mm, težine 220 grama je osigurana postupkom pasterizaicje u trajanju od 47 minuta. Pasterizacija je u komori sprovedena po definisanom programu termičke obrade koja podrazumeva izlaganje proizvoda delovanju zasićene vodene pare na temperaturi medijuma od 80°C. Nakon postupka toplotne obrade u istoj komori proizvod je hlađen u vremenu od 25 minuta. U svim mestima provere postignuta je temperatura od 74°C u termalnom centru (Kanali termokapla 2, 4, 5, 7, 8, 10 i 11), uz postizanje *pv* vrednosti u opsegu od 61,45 min (Kanal 10) do 81,07 min (Kanal 8). Postizanjem navedenih *pv* vrednosti validovana je temperatura od 74°C u termalnom centru fino usitnjenih barenih kobasica užeg dijametra, pakovanih u poliamidni omotač, kao adekvatna za osiguranje bezbednosti pod definisanim uslovima čuvanja hladnog lanca.

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Original scientific paper

Detection of *Campylobacter* spp. and hygiene indicators along the poultry slaughter line

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ABSTRACT

Campylobacter spp., a leading cause of foodborne disease, is closely associated with poultry meat. The slaughter line process involves numerous steps, which can contribute to cross-contamination with microorganisms. Our study aimed to assess the hygiene of the poultry slaughter process by determining levels of *Campylobacter* spp. and other relevant bacterial indicators of fecal contamination. Research was conducted in a medium-capacity poultry slaughter facility where most steps are automated. Sampling included broilers from two farms. Neck skin samples were collected for *Campylobacter* spp. analysis after both the defeathering and cooling processes. Additionally, swab samples for microbiological examination were taken from surfaces of both the defeathering machine and a meat-cutting table. Standard ISO methods were followed for quantitative microbiological analysis. The findings of *Campylobacter* spp. in neck skin and on surfaces that contact the carcasses were confirmed by PCR. Our findings reveal a strong correlation between the *Campylobacter* spp. counts on the neck skin and the levels of this pathogen detected on the tested surfaces. Furthermore, the aerobic bacteria count on the surfaces corresponds to both the *Enterobacteriaceae* count and the *Escherichia coli* count. A high degree of contamination with *Campylobacter* spp. (mean count in neck skin after cooling >3 $\log_{10}$ CFU/cm^2) and fecal contaminants (*Enterobacteriaceae* and *E. coli*) was detected in the examined poultry slaughterhouse. Therefore, the rules of good hygiene practice and hazard analysis and critical control point (HACCP) principles need to be reinforced in the facility with the aim of improving slaughter hygiene and product safety. The food business operator should review their food safety system, implement stricter hygiene measures in the facility, check the suppliers (farms and carriers) and apply good hygiene practices and biosecurity measures.

1. Introduction

In the European Union, according to data from the European Food Safety Agency (*EFSA*, 2023), campylobacteriosis is the most frequently registered foodborne disease, while according to data from the Institute for Public Health of Serbia, this disease is in second place in our country after salmonellosis (*IJZS*, 2020). Numerous vectors can contribute to the spread of *Campylobacter* spp. in the food chain, and one of the key ones is poultry meat (*EFSA* and *ECDC*, 2023). The process of slaughtering and processing poultry is complex and includes many process steps, which also affects the possibility of contamination and spread of bacteria during operations. To ensure appropriate process hygiene and product safety, strict adherence to good hygiene practices along with control measures based on the assessed risk or hazard analysis and critical control points (HACCP) principles is expected (*Althaus et al*., 2017). One of the key parameters for assessing the effectiveness of hygiene operations at slaughter is whether there is an increase or decrease in the number of microorganisms throughout the

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production process. Considering that poultry meat is often contaminated with *Campylobacter* spp., regulatory bodies have established a microbiological criterion (1000 CFU/g) for assessing the hygiene process of poultry slaughter (*EC*, 2005; *Serbia*, 2010). The objectives of this study were to examine the hygiene process of poultry slaughter by monitoring the number of *Campylobacter* spp., examining selected microbiological indicators (number of aerobic bacteria, number of *Enterobacteriaceae*, and number of *Escherichia coli*), and observing whether the number of tested bacteria shows a decreasing trend.

2. Materials and Methods

2.1. The Slaughterhouse and Slaughter Operations

The research was conducted in December 2023 at a medium-capacity poultry slaughterhouse (4000 broilers h^{-1}) where most of the processing steps are automated. After unloading, broilers were hung/shackled and then stunned with electric current, followed by automatic bleeding. Scalding was performed by immersion in a water tank at 50±1 °C, and was followed by automatic feather removal. After evisceration (manual) and final carcass processing, the carcasses were washed with cold water to remove visual contamination before cooling. The cooling process took place in a chamber at −1 °C to 0 °C with appropriate air circulation. The cooling process lasted for 3 h until the temperature in the carcass deep muscle reached ≤4 °C. Hygiene assessment at the slaughterhouse was carried out in accordance with standard operating procedures. The cleanliness assessment of the slaughter line was conducted using a scale (1 – clean, 2 – soiled, 3 – dirty) before the start of operations. The cleanliness of the slaughter line was rated as 1 before the start of operations. After completing the broiler slaughter from farm A, the slaughter line was thoroughly cleaned and washed before starting the broiler slaughter operations from farm B.

2.2 Farms of Origin and Transport

Broilers originated from two farms (farm A and farm B). The broilers were transported to the slaughterhouse using appropriate vehicles, with each cage containing 10 broilers. The total number of broilers transported from farm A was 2500, while 1600 were transported from farm B. The transport from farm A to the slaughterhouse took 30 minutes, and from farm B it took 90 minutes. The outside temperature at the slaughterhouse location was 2±1 °C with a relative humidity of 65%. There were no mortalities or injuries after transport. The age of the broilers was over 42 days (farm A – 48 days old; farm B – 49 days old). The mean broiler weight of live animals from farm A was 3.08 kg, and from farm B was 2.70 kg. The duration from arrival of the shipment at the slaughterhouse to the start of slaughter operations was 5 h. After unloading, in accordance with good hygienic practice, the transport vehicles were cleaned, washed and disinfected.

2.2. Sampling and Samples for Testing

Sampling for microbiological testing was conducted 30 min after the start of the slaughter operations. For the examination of the number of *Campylobacter* spp., neck skin samples were taken after the plucking/defeathering and cooling phases from a total of 36 slaughtered broilers (farm A – 18 carcasses and farm B – 18 carcasses). At each sampling point, 10 g of neck skin was taken from 9 carcasses, forming three composite samples (3×30 g). For microbiological testing of surfaces (*Campylobacter* spp., number of *Enterobacteriaceae*, number of aerobic bacteria, number of *E. coli*), a total of eight samples were taken (farm A – 4 swabs and farm B – 4 swabs). From each farm, two swabs were taken from the surfaces of the plucking machine and the carcass cutting table. The swab samples were taken from an area of 100 cm^2 that was in direct contact with the carcass surface, using the standard method (*Serbia*, 2018).

2.3. Microbiological Testing

The determination of the *Campylobacter* spp. count on broiler neck skin was performed using the standard method (*Serbia*, 2023). Briefly, each composite sample (3×10 g) was individually minced, and for the determination of *Campylobacter* spp., 10 g of the minced neck skin was homogenized with 90 mL of maximum recovery diluent (MRD, Oxoid). Further decimal dilutions were made from the primary dilution and inoculated onto appropriate microbiological media in accordance with the standard testing method (*Serbia*, 2023). For microbiological testing of swabs, 10 mL of MRD was added to each swab, from which further decimal

dilutions were made and inoculated onto appropriate microbiological media (Oxoid) in accordance with standard methods: *Campylobacter* spp., *Serbia* (2023); *Enterobacteriaceae* count, *Serbia* (2017); aerobic colony count, *Serbia* (2022); *E. coli* count, *Serbia* (2008).

2.4. Molecular Testing

From each plate, suspect colonies of *Campylobacter* spp. were transferred into a 1.5 mL tube containing 100 µL of phosphate buffer. After brief vortexing for 10 s, the tubes were placed in a thermal mixer with blocks (Thermo Fisher Scientific, USA) for complete microbial inactivation at 95 °C for 5 min. DNA isolation was conducted using a commercial kit for genomic DNA purification (GeneJET Genomic DNA Purification Kit*,* Thermo Fisher Scientific, USA). Polymerase chain reaction (PCR) was performed in a final volume of 25 µL, containing the following components: DreamTaq PCR Master Mix (2X) (Thermo Fisher Scientific, USA), 500 nM of each primer (Table 1) and 0.5 µL of isolated DNA. The PCR process involved initial denaturation for 5 min at 95 °C, followed by 40 cycles: 45 s at 94 °C, 45 s at 51 °C, and a final extension for 5 min at 72 °C. Electrophoresis of PCR products was conducted on a 2% agarose gel, with Midori Green Advance dye (Nippon Genetics, Japan), at 95 V for 45 min.

2.5. Statistical Analysis

Before any statistical analysis, the obtained data were tested for normality using the Shapiro-Wilk test ($p > 0.05$). Differences in microbiological parameters at the slaughter line between the two broiler origin farms were examined using an independent *t*-test. The estimation of correlation between the tested microbiological parameters was conducted using Pearson's correlation test. All values are expressed as mean±standard error. Statistical analysis of the results was performed using the SPSS 23 software package (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

The mean *Campylobacter* spp. counts in the neck skin of broilers along the slaughter line are presented in Table 2. In the neck skin samples taken immediately after the plucking phase, the number of *Campylobacter* spp. on broilers originating from farm A was significantly higher than the number on broilers originating from farm B. The reduction in *Campylobacter* spp. (log reduction from after plucking to after cooling) was 0.95±0.02 $\log_{10}$ CFU/g in the examined broilers from farm A, while this reduction was lower in broilers from farm B (Table 2). Although a reduction in the number of *Campylobacter* spp. was achieved during the cooling phase, as expected, due to the initially high number of these bacteria after the feather plucking phase, the number of these bacteria remained high and exceeded the critical limit (1000 CFU/g or 3.0 $\log_{10}$ CFU/g). According to *Althaus et al.* (2017), 29% of the examined poultry carcasses tested positive for *Campylobacter* spp., of which 42% had a bacterial count >3.0 $\log_{10}$ CFU/g. These authors found that the number of *Campylobacter* spp. decreases during the carcass scalding phase, but on average, the number of these bacteria increases (by 0.4 $\log_{10}$ CFU/g) after the plucking/defeathering phase. The risk of consumer infection increases with a higher number of bacteria contaminating the meat or product, emphasizing the importance of monitoring *Campylobacter* spp. in poultry meat production facilities. According to the current regulations in Serbia, out of 50 examined composite samples of poultry neck skin after cooling, no more than 15 examined composite samples should exceed the established limit (*Serbia*, 2010). Considering the importance of *Campylobacter* spp. for public health, this criterion will be tightened from 2025, and out of 50 examined samples, no more than 10 examined samples will be allowed to exceed the established threshold. If a food business operator (FBO) fails to meet the required criterion, the risk to public health increases, and it is the obligation of the FBO to review its system and implement corrective measures to verify and validate its food safety management procedures and good hygiene practices (*EFSA*, 2023).

Table 1. Primers used for the detection of the 16S rRNA region of *Campylobacter* spp.

The name of the primer	Sequence (5'–3')	Product size	Authors
16 S rRNA – F	ATCTAATGGCTTAACCATTAAAC	856 bp	*Dennis et al*., 1999
16 S rRNA – R	GGACGGTAACTAGTTTAGTAT		

Table 2. *Campylobacter* spp. counts (log_{10} CFU/g; mean±standard error) in the neck skin of broilers sampled on the slaughter line

	Farm A	Farm B	P-value
After plucking/defeathering	4.47±0.01	3.67±0.03	0.001
After cooling	3.52±0.03	3.12±0.12	0.05
Log reduction	0.95±0.02	0.56±0.1	0.08

In addition to examining the presence and count of *Campylobacter* spp. on the neck skin of broilers, the number of these microorganisms was also examined on surfaces that come into contact with the carcass/meat. By amplifying the 16S rRNA-specific region, the presence of *Campylobacter* spp. genomes was determined (Figure 1). Swab samples were taken from the surfaces of the defeathering machine and the carcass cutting table. The number of *Campylobacter* spp. on the examined surfaces of the feather plucking machine was significantly higher during the processing operations of broilers from farm A compared to farm B (Table 3). Considering that broilers from farm A had a significantly higher number of *Campylobacter* spp. in the examined neck skin samples (Table 2), greater contamination of the feather plucking machine surfaces during the processing of this group of animals is expected. No significant difference was found in the *Campylobacter* spp. count from the carcass cutting table during the slaughter of broilers from farm A and farm B.

Due to the complexity of operations and the relatively high level of automation in the poultry slaughter line, a large number of surfaces become contaminated during the process. *Campylobacter* spp. have the ability to form biofilms (*Laconi et al.*, 2023), and these can be a constant source of cross-contamination on the slaughter line. Biofilm forms after bacteria adhere to a surface and is significantly more difficult to remove than planktonic bacteria, so regular hygiene maintenance and constant removal of organic matter from the slaughter and processing line are of great importance, including washing and disinfection (*Araújo et al.*, 2022).

In addition to determining the presence of pathogenic microorganisms, the meat industry also monitors process hygiene indicators. For these investigations, the selected indicators are the numbers of aerobic bacteria, *Enterobacteriaceae* and *E. coli.* The number of aerobic bacteria generally indicates the number of bacteria and hygiene, while *Enterobacteriaceae* and *E. coli* are indicators of fecal contamination on the slaughter line (*Althaus et al.,* 2017). The number of aerobic bacteria found in surface swabs is shown in Table 4. A significantly higher number was found on the surfaces of the feather plucking machine during the slaughter phase of broilers from farm A compared to farm B. Significantly higher numbers of *Enterobacteriaceae* (Table 5) and *E. coli* (Table 6) were determined in surface swabs during the slaughter of broilers from farm A than of broilers from farm B, both on the surfaces of

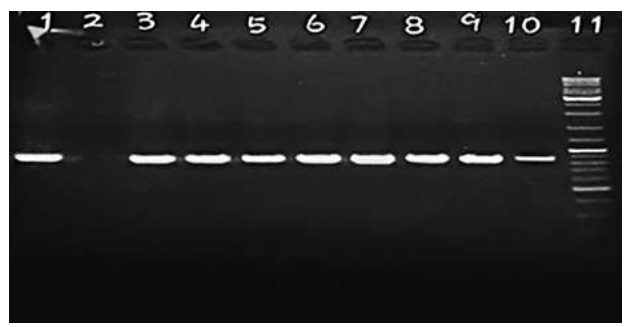

Figure 1. *Campylobacter* 16sRNA PCR test on agarose gel: (1) Positive control — *Campylobacter jejuni* ATCC 33560; (2) Negative control — water; (3–6) Samples from neck skin; (7–10) Swab samples; (11) DNA marker

Table 3. *Campylobacter* spp. counts (log_{10} CFU/g; mean±standard error) in swab samples from surfaces during the slaughter of broilers originating from different farms

	Farm A	Farm B	P-value
Plucking/defeathering machine	1.99±0.16	1.17±0.09	0.01
Meat-cutting table	0.52±0.13	0.84±0.11	0.16

Table 4. Aerobic bacteria counts (log_{10} CFU/cm^2; mean±standard error) in swab samples from surfaces during the slaughter of broilers originating from different farms

	Farm A	Farm B	P-value
Plucking/de-feathering machine	4.70±0.06	2.84±0.20	0.001
Meat-cutting table	3.93±0.04	3.42±0.08	0.006

Table 5. *Enterobacteriaceae* counts (log_{10} CFU/cm^2: mean±standard error) in swab samples from surfaces during the slaughter of broilers originating from different farms

	Farm A	Farm B	P-value
Plucking/de-feathering machine	2.08±0.18	0.84±0.18	0.009
Meat-cutting table	1.22±0.14	0.74±0.07	0.04

Table 6. *Escherichia coli* counts (log_{10} CFU/cm^2: mean±standard error) in swab samples from surfaces during the slaughter of broilers originating from different farms

	Farm A	Farm B	P-value
Plucking/de-feathering machine	1.48±0.26	0.30±0.16	0.02
Meat-cutting table	0.79±0.11	0.38±0.05	0.03

the feather/plucking machine and the carcass/meat cutting table.

Correlation analysis of the parameters investigated on the broiler slaughter line (Table 7) showed a high degree of correlation between the *Campylobacter* spp. count on the neck skin and the number of these bacteria on the slaughter line surfaces. Also, on the examined slaughter line surfaces, a high degree of correlation was measured between the aerobic bacteria count and the *Enterobacteriaceae* count. This correlation trend was observed in the case of the *E. coli* count, which was also directly correlated to the *Enterobacteriaceae* count on the slaughter line surfaces (Table 7).

Table 7. Correlation analysis of the parameters examined on broiler contact surfaces in the broiler slaughter line

	***Campylobacter* spp. count in neck skin**	***Campylobacter* spp. count from surface swab**	**Aerobic bacteria count from surface swab**	***Enterobacteriaceae* count from surface swab**
Escherichia coli count from surface swab	0.73**	0.63*	0.78**	0.96**
Enterobacteriaceae count from surface swab	0.85**	0.70*	0.78**	
Aerobic bacteria count from surface swab	0.64*	0.43		
Campylobacter spp. count from surface swab	0.83**			

* $p < 0.05$; ** $p < 0.01$

4. Conclusion

In this study, quantitative microbiological analysis was conducted of *Campylobacter* spp. on broiler carcasses (neck skin) and on selected surfaces in contact with the broiler carcass/meat. *Campylobacter* spp. were detected on the neck skin of the examined carcasses after both feather removal and carcass cooling, with the determined mean counts being >3 $\log_{10}$ CFU/g. Slaughter line surfaces were contaminated with both *Campylobacter* spp. and fecal indicator bacteria (*Enterobacteriaceae* and *E. coli*). In accordance with good hygiene practices and HACCP principles, in slaughter facilities where unacceptable bacterial contamination is measured, FBOs should reassess their food safety systems and implement enhanced hygiene measures in the facility, including supplier (farm) checks and adherence to good hygiene practices and biosecurity measures within the system. The aim is to reduce the presence of both *Campylobacter* spp. and bacterial indicators of process hygiene on slaughtered broiler carcasses.

Nalaz *Campylobacter spp.* i indikatora higijene na liniji klanja živine

Katarina Pavićević, Ivan Vićić, Milijana Stanojčić i Nedjeljko Karabasil

INFORMACIJE O RADU

Ključne reči:
Higijena
Brojleri
Hrana
Proces

APSTRAKT

Campylobacter spp. su vodeći uzročnici bolesti prenosivih hranom, a jedan od ključnih vektora je meso živine. Operacije klanja uključuju veliki broj procesnih koraka, što utiče i na mogućnost unakrsne kontaminacije mikroorganizmima. Naša studija ima za cilj da ispita higijenu procesa klanja živine putem praćenja broja *Campylobacter* spp. kao i ostalih odabranih mikrobioloških indikatora higijene procesa. Istraživanje je sprovedeno u objektu za klanje živine srednjeg kapaciteta, gde je većina procesnih koraka automatizovana. Uzorkovanjem su obuhvaćeni brojleri poreklom sa dve farme. Za ispitivanje broja *Campylobacter* spp. uzeti su uzorci kože vrata, nakon čerupanja i hlađenja. Za mikrobiološko ispitivanje površina *(Campylobacter* spp., broj enterobakterija, broj aerobnih kolonija, broj *E. coli*), uzeti su uzorci brisa sa površina mašine za čerupanje perja i stola za rasecanje mesa. Kvantitativna mikrobiološka analiza sprovedena je standardnim SRPS ISO metodama. Nalaz *Campylobacter* spp. na uzorcima kože vrata i brisevima površina potvrđen je PCR tehnikom. Može se konstatovati visok stepen korelacije nalaza broja *Campylobacter* spp. sa kože vrata i broja ovih bakterija sa ispitivanih uzoraka površina. Takođe, sa porastom nalaza broja aerobnih kolonija u ispitivanim uzorcima površina, može se primetiti i visok stepen korelacije porasta broja enterobakterija koji je u direktnoj vezi sa brojem *E. coli*. S obzirom da je u ispitivanom objektu za klanje živine, utvrđen visok stepen kontaminacije *Campylobacter* spp. i fekalnim kontaminentima (enterobakterije i *E. coli*) na trupovima i površinama, u skladu sa pravilima dobre higijenske prakse i principima HACCP, subjekat u poslovanju hranom treba da preispita sistem bezbednosti hrane i primeni pooštrene mere higijene pogona, uz proveru dobavljača (farme i prevoznici) i primenu principa dobre higijenske prakse i biosigurnosnih mera u sistemu sa ciljem unapređenja higijene klanja i bezbednosti proizvoda.

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Original scientific paper

Metal bioaccumulation in fish species from the Danube River in Serbia and evaluation of possible health risks

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ABSTRACT

The aim of the present study was to assess the content of metals in fish meat and to evaluate possible health risks from dietary consumption of fish caught from the Danube River in Serbia in the past fifteen years. Therefore, the metal pollution index (MPI) and the following health risk indexes were calculated: estimated daily intake (EDI), estimated weekly intake (EWI), % of provisional tolerable weekly intake (% PTWI), target hazard quotient (THQ), hazard index (HI), and target cancer risk (TR). Levels of Cd in common carp and Wels catfish from 2011 to 2013 and in silver carp in 2021 exceeded maximum allowed concentrations in fish meat. Wels catfish contained higher contents of Hg from 2011 to 2013 and Pb in 2010 than prescribed by the national regulation. Moreover, MPIs determined for common carp, Wels catfish, and barbel gradually decreased during the observed period, except for silver carp where a slight increasing trend was observed. The HI was higher than 1 in almost all studies, and exceeded maximum allowed levels prescribed by international and national regulations. In all presented studies, TR was lower than the acceptable lifetime risk (ARL) of 10^{-4}, except for As in common carp caught in Zemun and Grocka during 2013 when an unacceptable carcinogenic risk (> 10^{-4}) was detected (1.10 x10^{-4} and 1.43 x10^{-4}, respectively). It is necessary to implement regular monitoring of metal levels in fish from the Danube River in order to preserve human and environmental health.

1. Introduction

The increasing trend of metal pollution of the environment has gathered more attention in recent decades, since metals are toxic to both humans and environment (*Cordeli et al.*, 2023). Elements are divided into essential for living organisms and non-essential, but depending on concentration, both groups are toxic to organisms (*Milošković and Simić*, 2023). Metals are stable, non-biodegradable, and their levels have increased in recent decades due to industrial and agricultural activities (*Azar and Vajargah*, 2023). Once metals enter an aquatic environment, they do not degrade, but accumulate on solid surfaces or in aquatic organisms. After accumulation in a living organism, metals disrupt many physiological processes, leading to oxidative stress, alteration in cellular function, and impaired immune function (*Cordeli et al.*, 2023).

Waters in Serbia are loaded with different pollutants, and only 16% of wastewater is processed (*Milošković and Simić,* 2023). One of the waterways, the River Danube, is an international river and the second largest in Europe, flowing for about 588 km through Serbia. The largest part of the territory of the

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Republic of Serbia belongs to the Danube basin (about 92%) (*Official Gazette of the Republic of Serbia*, 2017).

Metal concentrations in the Danube River have increased in past decades (*Cordeli et al.,* 2023). Since the Danube River is important for commercial and recreational fishing (*Smederevac-Lalić et al.,* 2011), fish contaminated with metals could be harmful to human health. To preserve human and environmental health, it is necessary to regularly assess the metal contamination of water, sediment, and various fish species recommended as bioindicators for pollution of potential toxic elements (*Milošković and Simić*, 2023). Therefore, the aim of the present study was to assess the content of metals in fish meat and to evaluate possible health risks from dietary consumption of fish caught from the Danube River in Serbia in the past fifteen years.

2. Materials and Methods

2.1 Material

For calculation of health risks after fish consumption, metal levels in fish muscles were used from studies published in the past 15 years. The inclusion criteria were studies performed on fish species that are recommended as bioindicator species (common carp, Wels catfish, silver carp, and barbel) by *Milošković and Simić* (2023), research that investigated fish from the Danube River in Serbia in the past 15 years, and studies that determined levels of As, Cd, Cr, Cu, Fe, Hg, Pb, and Zn in fish muscles.

2.2. Methods

2.2.1. Metal pollution index (MPI)

The metal pollution index was used to assess the total level of metal accumulation in fish species according to *Usero et al.* (1997). MPI was calculated as the geometric mean of metal levels (As, Cd, Cr, Cu, Fe, Hg, Pb, and Zn) in fish muscle:

$$\text{MPI (mg/kg)} = (C_1 \times C_2 \times C_3 \ldots \text{x } C_n)^{1/n}$$

where C is the mean level of metal in fish muscle (as mg/kg of w.w.).

In some studies, metal levels were originally presented in dry weight, so these were then recalculated from dry to wet weight according to the following formula:

$$\text{Cww} = \text{Cdw} \times [(100-\%\text{H})]/100$$

where Cww is the metal content expressed as wet weight, Cdw is the metal content expressed as dry weight, and %H is the percentage of water in fish muscle (approximately 80%) (*USEPA*, 2010; *Subotić et al.,* 2021).

2.2.2. Health risk assessment

Health risk assessment was performed on studies that contain metal levels higher than prescribed by international and/or national regulations.

Estimation of daily intake rate (EDI)

The calculation of estimated daily intake (μg/kg of body weight (BW) per day) of As, Cd, Cr, Cu, Fe, Hg, Pb, and Zn for Serbian people was performed according to the *Griboff et al.* (2017):

$$(C\ element \times D\ food\ intake)\ /BW$$

where C, element, is the content of an element in fish muscle (as μg/kg of w.w.), D, food intake, is the average daily intake of fish by people in Serbia (20 g, *Janjić et al.,* 2015), and BW is the average body weight for adults (70 kg, *EFSA,* 2012).

Estimation of weekly intake rate (EWI) and percentage of provisional tolerable weekly intake (% PTWI)

Estimation of weekly intake rate was calculated according to the following equation:

$$\text{EWI} = EDI \times 7\ days$$

EWIs were compared with their respective provisional tolerable weekly intake (PTWI) rates. PTWI represents the provisionally allowed metal weekly intake that is determined for Cd (5.75 μg/kg BW per week), Cu (3500 μg/kg BW per week), Fe (5600 μg/kg BW per week), Hg (4 μg/kg BW per week), and Zn (7000 μg/kg BW per week) (*JECFA*, 2011). With respect to Pb and As, we used the withdrawn PTWI rates for Pb (25 μg/kg BW per week) and As (15 μg/kg BW per week), since new PTWI rates have not been established (*JECFA*, 2011). When EWI is lower than PTWI, consumption of the food does not pose a risk for human health. Percentage of provisional tolerable weekly intake (% PTWI) was calculated according to the following formula:

$$\%\ \text{PTWI} = \frac{EWI}{PTWI} \times 100$$

Target hazard quotient (THQ)

Target hazard quotient is a type of non-carcinogenic health risk assessment method and it was calculated according to *Ahmed et al.* (2015):

$$\text{THQ} = \frac{Efr \times ED \times FIR \times C}{RfD \times BW \times AT} \times 10^{-3}$$

where Efr is the exposure frequency (365 days a year), ED is the exposure duration (70 years, the average human life time), FIR is the fish ingestion rate (20 g

per day in Serbia), C is the average content of a metal in fish muscle (mg/kg of w.w.), RfD_o is the reference oral dose, i.e., an estimate of the daily exposure to which humans could be continually exposed during their lifetime without harmful effects to health. RfD_o for As, Cd, Cr, Cu, Fe, Hg, and Zn is 0.0001, 0.0003, 0.0001, 0.005, 0.003, 0.04, and 0.30 mg/kg of BW per day, respectively (*USEPA*, 2015), BW is the average body weight (for adult 70 kg), AT is the average time of non-carcinogenic exposure (365 days per year × number of exposure years, assuming 70 years). The reference oral dose for Pb was withdrawn by the *USEPA* (2015). Therefore, THQ for Pb was calculated according to *Jovic and Stankovic* (2014):

$$\mathrm{THQ} = \frac{C}{MRL}$$

where C is the detected Pb level in fish muscle (as mg/kg of w.w.), MRL is the maximum residue limit, set by Regulation (EC) No 2023/915, and in fish meat is 0.3 mg/kg of w.w. A THQ value of less than 1 implies that no evident risk will arise from fish consumption, while THQ higher than 1 poses a potential non-carcinogen risk to the exposed population.

Hazard index (HI)

Hazard index (HI) was calculated as the sum of THQ for all metals detected in fish muscle (*Li et al.,* 2013):

$$HI = \sum_{i=1}^{n} \mathrm{THQ}\,\mathrm{i}$$

where HI value lower than 1 indicates safe fish consumption, while HI ≥1 represents a hazard for consumers.

Target cancer risk (TR)

Target cancer risk evaluates the risk of possible development of cancer over a lifetime due to exposure to Cr, Pb, and As. Acceptable risk levels range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000) and are calculated according to following equation (*USEPA*, 2000):

$$\mathrm{TR} = \frac{Efr \times ED \times FIR \times C \times CSFo}{BW \times AT} \times 100^{-3}$$

where Efr is the exposure frequency (365 days per year), ED is the exposure duration (70 years, the average human life time), FIR is the fish ingestion rate (20 g, *Janjić et al.,* 2015), C is the average heavy metal level detected in fish muscle (mg/kg of w.w.), CSFo is the carcinogenic slope factor (Cr: 0.5 mg/kg/day; Pb: 8.5 $\times 10^{-3}$ mg/kg/day; As: 1.5 mg/kg/day, determined by the *USEPA* (2015)), BW is the average body weight (70 kg for adults), AT is the mean exposure period for the carcinogen (365 days per year × number of exposure years, assuming 70 years).

3. Results and discussion

3.1. Level of metals in fish meat from the Danube River in Serbia for the period 2010–2021

In Table 1, levels of metals detected in meat from fish caught in the Danube River from 2010 to 2021 are presented.

In common carp, levels of toxic elements ranged for As from 0.01 to 0.333 mg/kg w.w., for Cd from 0.001 to 0.082 mg/kg w.w., for Hg from 0.022 to 0.466 mg/kg w.w., and for Pb from 0.007 to 0.084 mg/kg w.w. In Wels catfish, heavy metal levels ranged for As from 0.0016 to 0.211 mg/kg w.w., for Cd from 0.0008 to 0.09 mg/kg w.w., for Hg from 0.0028 to 0.62 mg/kg w.w., and for Pb from 0.0012 to 1.58 mg/kg w.w. In silver carp, levels of As were from 0.0072 to 0.1968 mg/kg w.w., of Cd were 0.0028 to 0.0808 mg/kg w.w., of Hg were 0.012 to 0.16 mg/kg w.w., and of Pb were 0.003 to 0.14 mg/kg w.w. In barbel, levels of As in fish meat ranged from 0.189 to 0.314 mg/kg w.w., of Cd from 0.052 to 0.062 mg/kg w.w., of Hg from 0.054 to 0.325 mg/kg w.w., and of Pb from 0.022 to 0.062 mg/kg w.w.

The highest level of As was detected in common carp (0.333 mg/kg w.w.) in 2013 by *Jovanović et al.* (2017) and in barbel (0.314 mg/kg w.w.) in 2012 by *Morina et al.* (2016), but these levels did not exceed the maximum allowable level prescribed by international and national regulations (*FAO*, 1983; *FAO/WHO*, 1998; *WHO/FAO*, 2015; *Official Gazette of the Republic of Serbia*, 2019). Regarding Cd, higher levels than permissible were found in 2013 by *Jovanović et al.* (2017) in common carp (0.059 mg/kg w.w., 0.082 mg/kg w.w.) and in Wels catfish (0.068–0.069 mg/kg w.w.). Higher values of Cd than permissible were found in Wels catfish (0.09 mg/kg w.w.) during 2011–2013 (*Milošković et al.,* 2016) and in silver carp (0.0808 mg/kg w.w.) in 2021 (*Aleksić et al.,* 2025). The highest level of Hg was detected in common

Table 1. Metal bioaccumulation in fish meat from the Danube River in Serbia for period 2010–2021 and maximum allowed concentrations in fish meat (mg/kg w.w.) established by European Commission Regulation (*EU*, 2006), Codex Alimentarius Commission (*WHO/FAO*, 2015), *FAO* (1983), *FAO/WHO* (1998), and Serbian national regulation (*Official Gazette of the Republic of Serbia (OG RS)*, 81/2019) (*Cordeli et al.,* 2023; *Milošković and Simić,* 2023; *Aleksić et al.,* 2025)

As	Cd	Cr	Cu	Fe	Hg	Pb	Zn	Reference
-	0.05	-	-	-	0.5	0.3	-	European Commission Regulation (*EU*, 2006)
-	-	-	-	-	-	0.3	-	Codex Alimentarius Commission (*WHO/FAO*, 2015)
1.0	0.05	0.15–1.0	30	100	0.5	0.5	30	FAO (*FAO*, 1983)
-	0.5	-	30	-	0.5	0.5	40	FAO/WHO (*FAO/WHO*, 1998)
-	0.05	-	-	-	0.5	0.3	-	Serbian national regulation (*OG RS*, 81/2019)

As	Cd	Cr	Cu	Fe	Hg	Pb	Zn	Region	Year of sampling	Reference
Common carp (*Cyprinus carpio*)										
0.1262	0.0137	0.022	0.351	8.72	0.0223	0.0142	31.00	Novi Sad	2021	*Aleksić et al.* (2025)
0.1268	0.0132	0.023	0.213	4.92	0.0223	0.0165	26.90	Zemun	2021	*Aleksić et al.* (2025)
0.1007	0.0175	0.026	0.298	4.85	0.0240	0.0165	29.61	Grocka	2021	*Aleksić et al.* (2025)
0.01	0.01	-	-	-	0.240	0.048	-	Vinča	2013	*Ivanović et al.* (2016)
0.258	**0.059**	-	0.688	9.38	0.393	0.059	6.16	Zemun	2013	*Jovanović et al.* (2017)
0.333	**0.082**	-	0.757	9.68	0.466	0.084	6.17	Grocka	2013	*Jovanović et al.* (2017)
0.0026	0.0028	-	-	-	0.0414	0.007	-	Belgrade	2012	*Milanov et al.* (2016)*
0.132	0.001	0.002	0.26	3.924	0.178	-	11.80	Belgrade	2010	*Subotić et al.* (2013b)*
0.132	-	-	-	-	0.0468	-	10.85	Belgrade	2010	*Subotić et al.* (2013a)*
0.079	-	-	-	1.484	-	-	10.94	Belgrade	2010	*Lenhardt et al.* (2012)*
Wels catfish (*Silurus glanis*)										
0.0568	0.0080	0.0158	0.101	4.37	0.1160	0.0157	7.65	Novi Sad	2021	*Aleksić et al.* (2025)
0.0365	0.0102	0.0193	0.133	5.00	0.1390	0.0107	7.69	Zemun	2021	*Aleksić et al.* (2025)
0.0393	0.0115	0.0183	0.123	3.12	0.1750	0.0093	6.34	Grocka	2021	*Aleksić et al.* (2025)
0.161	**0.068**	-	1.55	8.32	0.208	0.058	7.06	Zemun	2013	*Jovanović et al.* (2017)
0.211	**0.069**	-	1.62	8.17	0.260	0.069	6.68	Grocka	2013	*Jovanović et al.* (2017)
0.10	**0.09**	0.145	0.07	0.95	0.33	0.17	7.62	Novi Sad	2011–2013	*Milošković et al.* (2016)
0.09	0.001	0.13	0.07	1.33	0.20	0.18	2.97	Zemun	2011–2013	*Milošković et al.* (2016)
0.11	0.004	0.14	0.07	0.55	**0.62**	0.16	3.00	Radujevac	2011–2013	*Milošković et al.* (2016)
0.0262	0.0008	0.0276	0.1898	3.892	0.3196	0.0012	3.92	Belgrade	2013	*Jovičić et al.* (2016)*
0.003	0.01	-	-	-	**0.53**	0.06	-	Vinča	2013	*Ivanović et al. (2016)*
0.0016	-	-	-	0.0654	0.0028	-	0.0016	Belgrade	2012	*Milanov et al.* (2016)*
-	-	-	-	-	-	**1.582**	-	Belgrade	2010	*Lenhardt et al.* (2012)*
Silver carp (*Hypophthalmichthys molitrix*)										
0.1968	0.0143	0.0358	0.391	21.08	0.0238	0.0315	10.11	Novi Sad	2021	*Aleksić et al.* (2025)
0.0852	0.0145	0.0305	0.639	8.44	0.0230	0.027	8.54	Zemun	2021	*Aleksić et al.* (2025)
0.1558	**0.0808**	0.0252	0.272	10.62	0.0120	0.0237	10.59	Grocka	2021	*Aleksić et al.* (2025)
0.0072	0.0028	-	-	-	0.028	0.0112	-	Belgrade	2012	*Milanov et al.* (2016)*
0.04	0.01	-	-	-	0.16	0.14	-	Vinča	2013	*Ivanović et al.* (2016)
-	-	-	-	2.51	-	0.003	6.38	Belgrade	2010	*Lenhardt et al.* (2012)*
Barbel (*Barbus barbus*)										
0.189	0.052	-	0.826	12.22	0.222	0.048	5.20	Zemun	2013	*Jovanović et al.* (2017)
0.239	0.062	-	0.839	11.91	0.325	0.062	6.02	Grocka	2013	*Jovanović et al.* (2017)
0.314	-	0.082	0.380	-	0.054	0.022	3.67	Belgrade	2012	*Morina et al.* (2016)*
0.280	-	-	-	-	-	-	2.58	Belgrade	2010	*Sunjog et al.* (2012)*

Legend: *In studies by *Lenhardt et al.* (2012), *Sunjog et al.* (2012), *Subotić et al.* (2013a), *Subotić et al.* (2013b), *Jovičić et al*. (2016), *Morina et al.* (2016), *and Milanov et al.* (2016), metal levels were originally presented in μg/g d.w., so in the current study, these levels were recalculated from dry to wet weight according to the following formula: $Cww = Cdw \times \frac{(100-\%H)}{100}$, where Cww is the metal content expressed as wet weight, Cdw is the metal content expressed as dry weight, %H is the percentage of water in fish muscle (approximately 80%) (*USEPA*, 2010; *Subotić et al.,* 2021)

carp (0.393–0.466 mg/kg w.w.) in 2013 (*Jovanović et al.,* 2017), while higher levels of Hg than permissible were detected in Wels catfish during 2011–2013 (0.62 mg/kg w.w.) by *Milošković et al.* (2016) and in 2013 (0.53 mg/kg w.w.) by *Ivanović et al.* (2016). Regarding Pb, a higher level than permissible was detected in Wels catfish (1.582 mg/kg w.w.) in 2010 by *Lenhardt et al.* (2012).

3.2. The metal pollution index (MPI)

The metal pollution index (MPI) of four fish species (common carp, Wels catfish, silver carp, and barbel) caught from the Danube River in Serbia from 2010 to 2021 is presented in Figure 1.

MPI ranged from 0.007 to 1.086 in common carp, from 0.005 to 1.582 in Wels catfish, from 0.009 to 0.364 in silver carp, and from 0.190 to 0.850 in barbel. The highest values of MPI were observed in 2010, then decreased during 2012, and thereafter increased in 2013. Moreover, MPI determined for common carp, Wels catfish, and barbel gradually decreased during the observed period, except for silver carp where a slight increasing trend was noticed. Since the content of metals in the meat of the four fish species was not measured from 2013 to 2021, this lack of information cannot provide a reliable conclusion about the trends of metal levels in meat from fish caught in the Danube River. *Subotić et al.* (2013a) found that MPI in 2010 ranged from 0.840 (Wels catfish) to 1.140 (common carp), emphasizing that MPI was higher in omnivorous than in carnivorous fish species. Similarly, *Aleksić et al.* (2025) reported the highest MPI was in silver carp (herbivorous), followed by the MPI in common carp (omnivorous), while the lowest MPI was in Wels catfish (carnivorous), due to its different feeding behaviour. *Milošković et al.* (2016) pointed out that MPI is reliable indicator of metal contamination of fish.

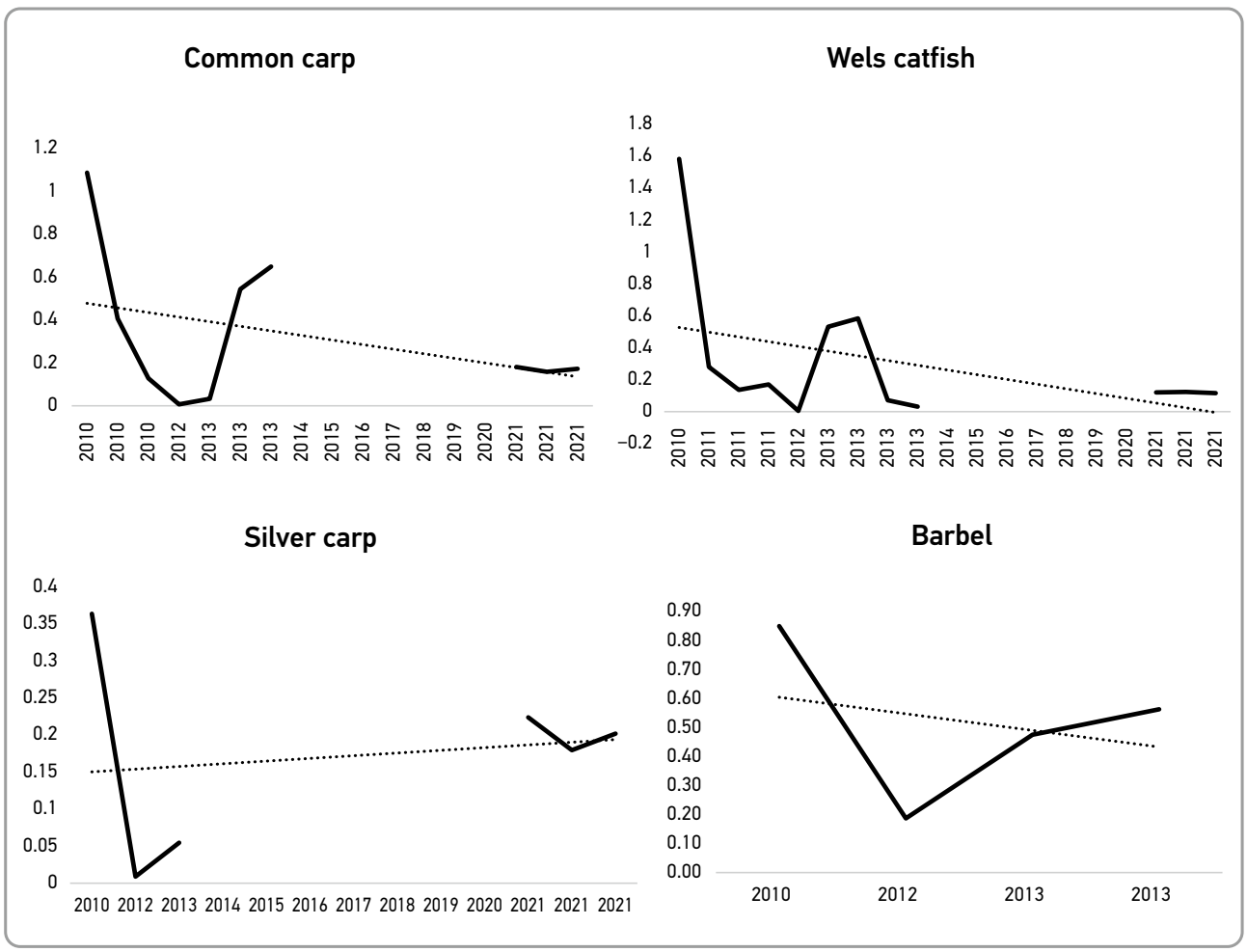

Figure 1. The metal pollution index (MPI) of four fish species (common carp, Wels catfish, silver carp, and barbel) from the Danube River in Serbia for period 2010-2021 (geometric mean of As, Cd, Cr, Cu, Fe, Hg, Pb, and Zn levels)

3.3. Estimation of daily intake rate (EDI), weekly intake rate (EWI), and percentage of provisional tolerable weekly intake (% PTWI)

Table 2 presents the EDI, EWI, and % PTWI for meat of fish collected from the Danube River in Serbia, 2010–2021.

The data are from studies that reported the maximum allowed levels for metals in fish meat had been exceeded. The EDI and EWI rates were presented as µg/kg of BW per day and week, respectively. The highest EDI rate for As was found in common carp (0.0912 µg/kg BW per day) by *Jovanović et al.* (2017), for Cd (0.0247 µg/kg BW per day) and Hg (0.1699 µg/kg BW per day) in Wels catfish by *Milošković et al.* (2016), and for Pb in Wels catfish (0.4334 µg/kg BW per day) by *Lenhardt et al.* (2012). EWI rates were seven times greater than EDI rates, and followed the same pattern as EDI

Table 2. EDI (µg/kg BW per day), EWI (µg/kg BW per week), % PTWI, THQ, and TR for fish meat from the Danube River in Serbia for the period 2010-2021 (presented data are from studies in which the maximum allowed metal levels in fish meat were exceeded)

	As	Cd	Cr	Cu	Fe	Hg	Pb	Zn	Region	Year of sampling	Reference
Common carp (*Cyprinus carpio*)											
EDI	0.0707	0.0162	-	0.1885	2.57	0.1077	0.0162	1.69	Zemun	2013	*Jovanović et al.* (2017)
EWI	0.4948	0.1132	-	1.32	17.99	0.7537	0.1132	11.81			
% PTWI	3.30	1.97	-	0.0377	0.3212	18.84	0.4526	0.1688			
THQ	0.2457	0.1686	-	0.0049	0.0038	1.12	0.1967	0.0059			
TR	1.11E-04	-	-	-	-	-	1.43E-07	-			
EDI	0.0912	0.0225	-	0.2074	2.65	0.1277	0.0230	1.69	Grocka	2013	*Jovanović et al.* (2017)
EWI	0.6386	0.1573	-	1.45	18.56	0.8937	0.1611	11.83			
% PTWI	4.26	2.74	-	0.0415	0.3315	22.34	0.6444	0.1690			
THQ	0.3171	0.2343	-	0.0054	0.0040	1.3314	0.2800	0.0059			
TR	1.43E-04	-	-	-	-	-	2.04E-07	-			
Wels catfish (*Silurus glanis*)											
EDI	-	-	-	-	-	-	0.4334	-	Belgrade	2010	*Lenhardt et al.* (2012)
EWI	-	-	-	-	-	-	3.03	-			
% PTWI	-	-	-	-	-	-	12.14	-			
THQ	-	-	-	-	-	-	5.27	-			
TR	-	-	-	-	-	-	3.84E-06	-			
EDI	0.0441	0.0186	-	0.4247	2.28	0.0570	0.0159	1.93	Zemun	2013	*Jovanović et al.* (2017)
EWI	0.3088	0.1304	-	2.97	15.96	0.3989	0.1112	13.54			
% PTWI	2.06	2.27	-	0.08	0.28	9.97	0.44	0.19			
THQ	0.1533	0.1943	-	0.0111	0.0034	0.5943	0.1933	0.0067			
TR	6.90E-05		-	-	-	-	1.41E-07	-			
EDI	0.0578	0.0189	-	0.4438	2.2384	0.0712	0.0189	1.83	Grocka	2013	*Jovanović et al.* (2017)
EWI	0.4047	0.1323	-	3.11	15.67	0.4986	0.1323	12.81			
% PTWI	2.70	2.30	-	0.0888	0.2798	12.47	0.5293	0.1830			
THQ	0.2010	0.1971	-	0.0116	0.0033	0.7429	0.2300	0.0064			

	As	Cd	Cr	Cu	Fe	Hg	Pb	Zn	Region	Year of sampling	Reference
Wels catfish (*Silurus glanis*)											
TR	9.04E-05	-	-	-	-	-	1.68E-07	-			
EDI	0.0274	0.0247	0.0397	0.0192	0.2603	0.0904	0.0466	2.09	Novi Sad	2011-2013	*Milošković et al.* (2016)
EWI	0.1918	0.1726	0.2781	0.1342	1.82	0.6329	0.3260	14.61			
% PTWI	1.28	3.00	1.8539	0.0038	0.0325	15.82	1.30	0.2088			
THQ	0.0952	0.2571	0.0138	0.0005	0.0004	0.9429	0.5667	0.0073			
TR	4.29E-05	-	2.07E-05	-	-	-	4.13E-07	-			
EDI	0.0301	0.0011	0.0384	0.0192	0.1507	0.1699	0.0438	0.8219	Radujevac	2011–2013	*Milošković et al.* (2016)
EWI	0.2110	0.0077	0.2685	0.1342	1.05	1.19	0.3068	5.75			
% PTWI	1.41	0.1334	1.79	0.0038	0.0188	29.73	1.23	0.0822			
THQ	0.1048	0.0114	0.0133	0.0005	0.0002	1.77	0.5333	0.0029			
TR	4.71E-05		2.00E-05	-	-	-	3.89E-07	-			
EDI	0.0008	0.0027	-	-	-	0.1452	0.0164	-	Vinča	2013	*Ivanović et al.* (2016)
EWI	0.0058	0.0192	-	-	-	1.02	0.1151	-			
% PTWI	0.0384	0.3335	-	-	-	25.41	0.4603	-			
THQ	0.0029	0.0286	-	-	-	1.51	0.2000	-			
TR	1.29E-06	-	-	-	-	-	1.46E-07	-			
Silver carp (*Hypophthalmichthys molitrix*)											
EDI	0.0427	0.0221	0.007	0.0745	2.91	0.0033	0.0065	2.90	Grocka	2021	*Aleksić et al.* (2025)
EWI	0.2988	0.1550	0.048	0.5216	20.37	0.0230	0.0455	20.31			
% PTWI	1.99	2.69	0.32	0.01	0.36	0.58	0.18	0.29			
THQ	0.1484	0.2309	0.0024	0.0019	0.0043	0.0343	0.0790	0.0101			
TR	6.68E-05	-	3.60E-06	-	-	-	5.76E-08	-			

rates. Moreover, EWI rates were lower than PTWI rates set by *JECFA* (2011) and for As and Cd, did not exceed 5%, indicating a low health risk for population. However, % PTWI for Pb was higher than 10% in Wels catfish (12.14%) (*Lenhardt et al.*, 2012), while for Hg, % PTWIs were even higher than 20%, representing a moderate health risk (*Alvarado et al.,* 2021). Determined % PTWIs for Hg were 22.34% in common carp found by *Jovanović et al.* (2017), and 25.41% and 29.73% in Wels catfish found by *Ivanović et al.* (2016) and *Milošković et al.* (2016), respectively. Since in all observed studies from 2010 to 2021, the content of Hg in fish meat from the Danube River exceeded 20 µg/kg of w.w., according to the International Commission for the Protection of the Danube River, the Danube River is not of good ecological status (*ICPDR*, 2021**).** Therefore, it is necessary to regularly monitor Hg levels and limit fish consumption when needed, especially for children and pregnant women (*Alvarado et al.,* 2021).

3.4. Target hazard quotient (THQ), hazard index (HI), target cancer risk (TR)

Table 2 presents THQ and TR, while Figure 2 shows HI for meat from fish collected from the Danube River in Serbia during 2010–2021.

THQ and HI represent the risk of non-carcinogenic effects of ingested metals from fish meat, and THQ decreased in the following order, Pb > Hg > As > Cd > Cr > Cu > Zn > Fe. THQs for all analysed metals were lower than 1, except for Pb in Wels catfish (5.27) determined by *Lenhardt et al.* (2012), Hg in common carp (1.12 and 1.33) found by

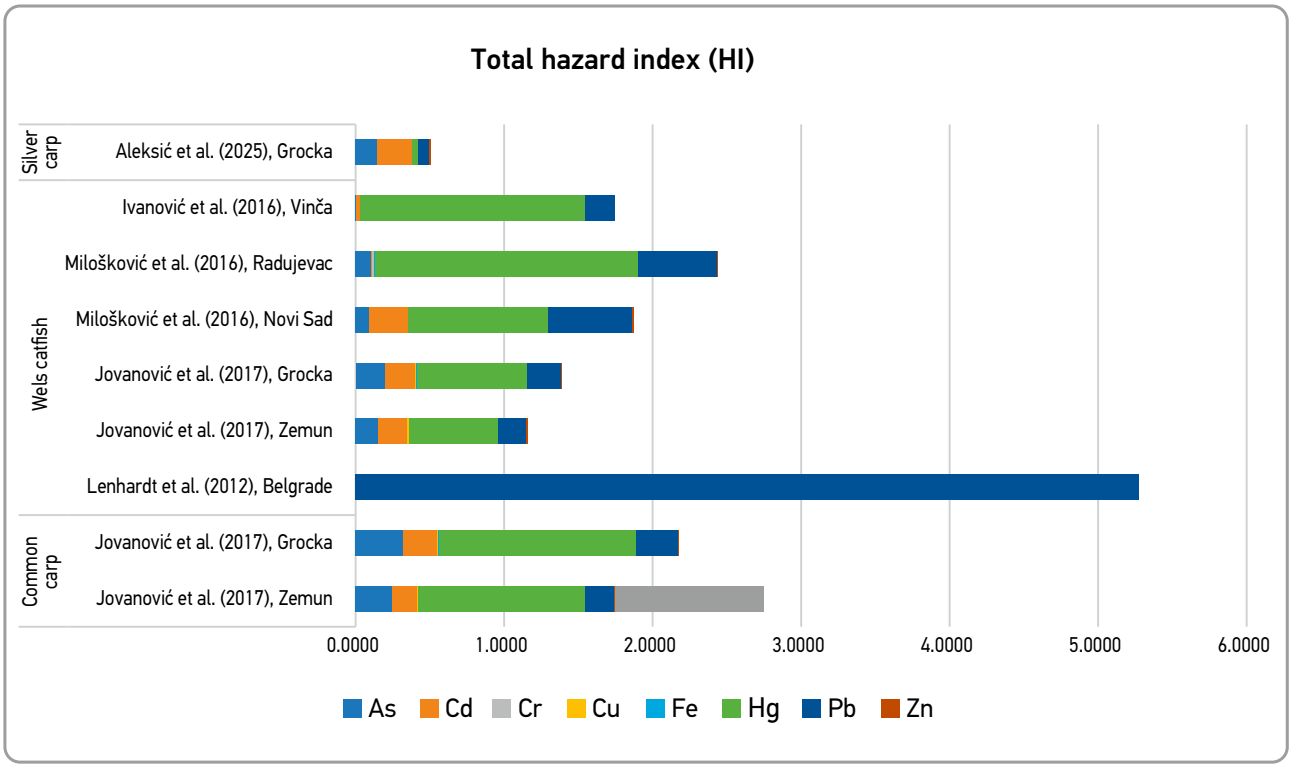

Figure 2. Estimated total hazard index (HI) from consumption of fish from the Danube River in Serbia for the period 2010–2021 (presented data are from studies in which the maximum allowed metal levels in fish meat were exceeded)

Jovanović et al. (2017), and Hg in Wels catfish found by *Milošković et al.* (2016) (1.77) and *Ivanović et al.* (2016) (1.51). In those studies where THQ values were higher than 1, consuming a fish from the Danube River posed a significant risk for human health. Similarly, HI was higher than 1 in all studies, and so exceeded the maximum allowable level prescribed by international and national regulations, except for one study (*Aleksić et al.*, 2025) where HI was 0.5089. In all other studies, HI ranged from 1.1564 to 5.2733, being the highest in Wels catfish found by *Lenhardt et al.* (2012). In those previously mentioned studies with HI > 1, consuming a fish represented a hazard for consumers.

Regarding the risk from developing cancer after consuming a fish from the Danube River, TR of the analysed studies ranged for As from 1.29E-06 to 1.43E-04, for Cr from 3.60E-06 to 2.07E-05, and for Pb from 5.76E-08 to 3.84E-06. Regarding As and Cr, TR was lower than the acceptable lifetime risk (ARL) of 10^{-4}, except for As in common carp caught in Zemun and Grocka during 2013, when an unacceptable carcinogenic risk ($\geq 10^{-4}$) was detected (1.10×10^{-4} and 1.43×10^{-4}, respectively) by *Jovanović et al.* (2017). The carcinogenic risk for Pb was lower than 10^{-6} and recognized as negligible in most studies, except for in Wels catfish (3.84E-06) caught in 2010, when TR was regarded as acceptable (*Lenhardt et al.,* 2012).

4. Conclusion

Higher levels of toxic elements than permissible were found for Cd in common carp and Wels catfish during 2011–2013 and in silver carp in 2021. Higher levels of Hg than permissible were detected in Wels catfish from 2011 to 2013. The content of Pb exceeded the maximum allowed concentration in Wels catfish in 2010. MPIs determined for common carp, Wels catfish, and barbel gradually decreased during the observed period, except for silver carp, where a slight increasing trend was noticed. Determined % PTWIs for Hg were higher than 20% in common carp and Wels catfish during 2011–2013, representing a moderate health risk. THQs for all analysed metals were generally lower than 1, except for Pb in Wels catfish in 2010, for Hg in common carp and for Hg in Wels catfish during 2011–2013. Moreover, HI was higher than 1 in almost all studies, so this exceeded the maximum allowable level prescribed by international and national regulations, indicating a hazard for consumers.

Carcinogenic risk in all analysed studies was lower than the acceptable lifetime risk (ARL) of 10^{-4}, except for As in common carp caught during 2013. Since there are no published data concerning measured toxic elements in fish meat from the Danube River from 2013 to 2021, it is necessary to implement regular monitoring of metal levels in fish from the Danube River and to limit fish consumption when needed, especially for children and pregnant women.

Bioakumulacija metala u ribama izlovljenih iz reke Dunav i procena mogućih zdravstvenih rizika

Marija Starčević, Nenad Katanić, Milica Laudanović, Dragoljub Jovanović, Aleksandra Tasić, Branislav Baltić i Nataša Glamočlija

INFORMACIJE O RADU

Ključne reči:
Reka Dunav
Šaran
Som
Tolstolobik
Mrena

APSTRAKT

Cilj ovog rada je bio da se utvrdi sadržaj metala u mesu riba i procene mogući zdravstveni rizici nakon konzumacije ribe izlovljene iz reke Dunav u Srbiji u poslednjih petnaest godina. Zbog toga su izračunati indeks zagađenosti mesa riba metalima (Metal pollution index — MPI), procenjena dnevna stopa unosa metala (Estimated daily intake rate — EDI), procenjena nedeljna stopa unosa metala (Estimated weekly intake rate — EWI), procenat privremenog podnošljivog nedeljnog unosa metala (% of provisional tolerable weekly intake — % PTVI), koeficijent opasnosti od određenog metala (Target hazard quotient — THQ), ukupni rizik štetnosti od metala (Hazard index — HI) i rizik od nastanka raka nakon konzumacije ribe (Target cancer risk — TR). Sadržaj kadmijuma (Cd) u šaranu i somu od 2011. do 2013. godine i u tolstolobiku 2021. godine bio je veći od maksimalno dozvoljene vrednosti za meso riba. U mesu soma zapažen je veći sadržaj žive (Hg) od 2011. do 2013. godine i olova (Pb) u 2010. godini nego što je dozvoljeno nacionalnim propisom. Pored toga, utvrđen MPI za šarana, soma i mrenu postepeno se smanjivao tokom posmatranog perioda, osim kod tolstolobika gde je uočen blagi trend rasta. Utvrđeno je da je HI indeks veći od 1 u skoro svim studijama koje su premašile maksimalno dozvoljene nivoe metala propisane međunarodnim i nacionalnim propisima. U svim prikazanim studijama TR je bio niži od prihvatljivog životnog rizika od 10^{-4}, osim za arsen (As) kod šarana izlovljenog u Zemunu i Grockoj tokom 2013. godine kada je otkriven neprihvatljiv kancerogen rizik ($> 10^{-4}$) (1.10×10^{-4} i 1.43×10^{-4}, redom). Stoga, neophodno je redovno pratiti sadržaj metala u ribama izlovljenih iz Dunava u cilju očuvanja zdravlja ljudi i životne sredine.

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Original Scientific Paper

Assessing the carbon footprint of cheese production: A study on mass and nutritional indicators

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ABSTRACT

Cheese production involves various processes, with milk production contributing over 85% of the overall environmental impact. This study used a simplified life cycle assessment to estimate the carbon footprint of 13 cheese varieties based on milk quantity (1 L of raw cow milk emits 1 kg CO2e). Results were presented in relation to cheese mass and nutritional values (protein, fat, energy). Related to cheese mass, Parmesan had the highest carbon footprint (16.40 kg CO2e/kg), which correlated to milk quantity. However, when nutritional values were used as functional units, ricotta showed the highest carbon footprint for protein (88.62 kg CO2e/kg), and cottage cheese for fat (157.18 kg CO2e/kg) and energy (1.48 kg CO2e/1000 kJ). Spearman correlation coefficients for carbon footprint confirmed the correlations between the nutritional values ($p<0.05$), but no correlation was found between carbon footprint and cheese mass ($p>0.05$). Promoting nutritional values as functional units could encourage consumer alignment of dietary choices with sustainability goals.

1. Introduction

Food life cycle assessment (LCA) studies have traditionally used mass- or volume-based functional units (FUs) for expressing measured environmental impacts (*Djekic, Pojić, et al.*, 2019). However, recent research has increasingly assessed both nutritional and environmental dimensions simultaneously, thereby highlighting nutritional LCA (n-LCA) as a promising direction (*Green, Nemecek, & Mathys*, 2023). The choice between standard LCA and n-LCA is currently the subject of intense debate.

Carbon footprint, often one of the key metrics calculated within an LCA, is mainly associated with the emission of greenhouse gasses, and is expressed through measuring the global warming potential (GWP) (*ISO*, 2018).

Using mass as the FU for calculating the carbon footprint leads to a simple interpretation, but does not capture nutrition. The advantage of using protein content as a FU is the ability to simplify environmental impact comparisons between products with high nutritional value, such as cheese, and dairy alternatives, such as tofu. Energy content, which is commonly used in nutrition science to calculate dietary guidelines, can help connect issues related to obesity and overconsumption with the environmental impact. As defined in ISO 14040, (*ISO*, 2006), and highlighted by *McLaren et al.* (2021), the selection of a FU depends on the specific purpose and scope of the study.

Different cheese types differ greatly in the amount of milk used for their production, which is measured in terms of cheese yield (*Hill & Ferrer*, 2021), but also in terms of the cheeses' nutritional values (*O'Brien & O'Connor*, 2004). Europe is the world's largest cheese producer, with cheese being the most widely produced dairy product. Since the abolition of the milk quota system in 2015, the production of cheese has been steadily

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increasing (*Finnegan, Yan, Holden, & Goggins*, 2018). In line with this expanding cheese production, it is crucial to study the associated environmental impacts.

Up to now, the environmental impact of cheese production has been assessed through various case studies, such as mozzarella cheese production (*Palmieri, Forleo, & Salimei*, 2017), Romanian sheep cheese production (*Ghinea & Leahu*, 2023) or LCA of different dairy products, including cheese, in Serbia (*Djekic, Miocinovic, Tomasevic, Smigic, & Tomic*, 2014).

There is also increasing data on the carbon footprint of cheese production as calculated using nutritional indicators as FUs, exemplified by the study on mature Gouda (*McLaren et al.*, 2021). In cheese production, considering the process from cradle to factory gate, milk production is the most significant contributor to GWP (79–95%), followed by the acidification potential (88–99%), and eutrophication potential (59–99%) (*Finnegan et al.*, 2018).

Considering the fact that the volume of raw milk required for cheese production differs greatly with the type of cheese, from 5.3 kg/kg of fromage frais (*Domagała et al.*, 2020) to 16.4 kg/kg of Parmesan (*Hill & Ferrer*, 2021), the aim of this study was to investigate the impact of five different FUs, i.e., both nutritional and mass-based parameters, on the calculated carbon footprint of a wide range of cheese varieties.

It is evident that there is no single FU meant to be universally applied. Each FU presents distinct benefits and drawbacks in the interpretation of the cheese's environmental impact. By presenting and analyzing the carbon footprints based on various FUs and various cheese types, we aim to provide a comprehensive carbon footprint matrix that can inform future LCA studies and guide decision-making in the cheese production and consumption segment.

2. Materials and Methods

The main LCA stages are defined by the international standard for this type of study (*ISO*, 2006). For the purpose of this study, a partial, simplified LCA was employed, applying the following criteria: (i) the goal of this LCA was to calculate and compare carbon footprint of 13 types of cheese; (ii) FUs used to express the carbon footprint were mass (kg) and four nutritional values (protein, fat, energy and calcium); (iii) inventory analysis of raw milk production was based on calculation that included the quantity of milk used for cheese production; (iv) impact assessment covered only one environmental impact, i.e, the GWP; (iv) the interpretation focused on understanding how the quantity of milk affects the calculation of the cheese's carbon footprint when different FUs are used.

Table 1. Quality parameters of 13 different cheese types

Cheese type	Yield (kg milk/kg cheese)	Total solids (%)[a]	Protein (%)[a]	Fat (%) [a]	Calcium (mg/100g)[f]	Energy	
						kcal[a]	kJ[a]
Brie	7.1[e]	51.4	19.3	26.9	540	319	1,323
Camembert	6.8[e]	49.3	20.9	23.7	350	297	1,232
Cheddar	10.0[e]	64.0	25.5	34.4	720	412	1,708
Cottage	6.1[c]	20.9	13.8	3.9	73	98	413
Edam	11.5[e]	56.2	26.0	25.4	770	333	1,382
Emmental	11.0[e]	64.3	28.7	29.7	970	382	1,587
Feta	7.1[e]	43.5	15.6	20.2	360	250	1,037
Fromage frais	5.3[d]	22.3	6.8	7.1	89	113	469
Gouda	10.3[e]	59.9	24.0	31.0	740	375	1,555
Gruyere	11.5[e]	65.0	27.2	33.3	950	409	1,695
Mozzarella	9.0[e]	50.2	25.1	21.0	590	289	1,204
Parmesan	16.4[e]	81.6	39.4	32.7	1,200	452	1,880
Ricotta	8.3[b]	27.9	9.4	11.0	240	144	599

Source of data: [a] (*O'Brien & O'Connor*, 2004), [b] (*Ortiz Araque, Darré, Ortiz, Massolo, & Vicente*, 2018) [c] (*Klei, et al.*, 1998) [d] (*Domagała, et al.*, 2020) [e] (*Hill & Ferrer*, 2021) [f] (*O'Brien & O'Connor*, 2004)

For the purpose of this study, it was assumed that 1 kg of CO_2 is emitted into the atmosphere for each kg of raw milk produced, as proposed in the literature (*IDF*, 2009, 2023). Table 1 shows the quality parameters of 13 different cheese types. Data were extracted from literature sources (*Domagała et al.*, 2020; *Hill & Ferrer*, 2021; *Klei et al.*, 1998; *O'Brien & O'Connor*, 2004; *Ortiz Araque, Darré, Ortiz, Massolo, & Vicente*, 2018)

The impact of processing factors (such as energy or water) for cheese production are below 5%, as outlined in LCA databases (*openLCA*, 2024), and were not considered in this calculation.

The Spearman rank order correlation coefficient (rs) was calculated to measure the correlation between the carbon footprints of the 13 different types of cheese expressed in the selected FUs.

3. Results and Discussion

The findings revealed that presenting the carbon footprint as kg CO_{2e}/kg of cheese highlighted Parmesan as the cheese having the highest carbon footprint (16.40 kg CO_{2e}/kg of cheese). In general, as a result of the simplified calculation of GWP, the environmental impact of cheese production using cheese mass as a FU is directly correlated with the quantity of cheese milk. However, employing nutritional values as FUs revealed that among the 13 cheeses, ricotta exhibited the highest carbon footprint when it comes to protein (88.62 kg CO_{2e}/kg of protein), while cottage cheese had the highest GWP related to both fat content and energy (157.18 kg CO_{2e}/kg of fat and 1.48 kg CO_{2e}/1000 kJ, respectively) (Table 2).

In the study by *Katz-Rosene, Ortenzi, McAuliffe, and Beal* (2023) the term "cheese" was used in the context of LCA. However, the present study suggests that greater precision in defining "cheese" could improve the clarity and accuracy of such evaluations, given the considerable variation in the carbon footprints among the different types of cheese, especially when combined with the various FUs (Table 2).

According to the literature in which mass indicators were used as the FU, fresh cheeses could have lower environmental impacts than do semi-hard or hard cheeses (*Finnegan et al.*, 2018). However, the current study reveals that when protein content is considered as the FU, the GWP of fromage frais or ricotta is nearly double that of Edam or Gouda. This difference is even more pronounced when calcium content is considered as the FU. For instance, in that case, the GWP of cottage cheese is up to seven times higher than that of Gruyere (Table 2).

When GWP is calculated in relation to mass as a FU, a boundary was set (*Röös, Ekelund, & Tjärnemo*, 2014) at the threshold at 4 kg CO_{2e} for the transition from green to yellow label. The next

Table 2. Carbon footprint of different types of cheeses expressed in different functional units

Cheese type	kg CO_{2e}/ kg cheese	kg CO_{2e}/ 1 kg protein	kg CO_{2e}/ 1 kg fat	kg CO_{2e}/ 1000 KJ	kg CO_{2e}/ 1000 mg Ca)
Brie	7.10	36.79	26.39	0.54	1.31
Camembert	6.80	32.54	28.69	0.55	1.94
Cheddar	10.10	39.61	29.36	0.59	1.40
Cottage cheese	6.13	44.42	157.18	1.48	8.40
Edam	11.50	44.23	45.28	0.83	1.49
Emmental	11.00	38.33	37.04	0.69	1.13
Feta	7.10	45.51	35.15	0.68	1.97
Fromage frais	5.32	78.24	74.93	1.13	5.98
Gouda	10.30	42.92	33.23	0.66	1.39
Gruyere	11.50	42.28	34.53	0.68	1.21
Mozzarella	9.00	35.86	42.86	0.75	1.53
Parmesan	16.40	41.62	50.15	0.87	1.37
Ricotta	8.33	88.62	75.73	1.39	3.47

Table 3. Spearman's Rho correlation coefficient between carbon footprints of different types of cheese expressed in five functional units

Different functional units	Cheese mass	Protein	Fat	Energy	Calcium
Cheese mass	1.000	−0.182	−0.074	−0.055	−0.733**
Protein	−0.182	1.000	0.654*	0.644*	0.544
Fat	−0.074	0.654*	1.000	0.999**	0.544
Energy	−0.055	0.644*	0.999**	1.000	0.523
Calcium	−0.733**	0.544	0.544	0.523	1.000

*. Correlation is significant at the 0.05 level (2-tailed).

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

threshold was set at 14 kg CO_{2e} as a transition from yellow to red. *Rysselberge and Röös* (2021) reported that all cheeses fall within the yellow range. The current study confirms these data, with the exception of Parmesan, which was in the red range. In parallel, all protein-rich products, such as lentils, dry soybeans, and tofu, are consistently in the green range, i.e., below the 4 kg CO_{2e} threshold, when assessed via the mass indicator approach. Based on this idea, it can be claimed that all types of cheese have a higher negative impact on the environment and carbon footprint than plant-based alternatives (*Shabir et al.*, 2023).

However, a shift to the nutrient or micronutrient approach yields contrasting results. For instance, while the global average carbon footprint of cheese stands approximately eight times higher than that of tofu per kilogram of retail weight, this difference narrows significantly to about 1.8 times when recalculated using the targeted priority micronutrient value (*Katz-Rosene et al.*, 2023). Table 3 shows the correlation of GWP with the different FUs. GWP expressed as cheese mass was correlated with GWP expressed as calcium content, while the protein-related GWP was correlated with fat- and energy-related GWP.

Despite the limited presence of carbon footprint labels in the market, it could be agreed that they play a crucial role in enabling consumers to make informed decisions that contribute to addressing climate change (*Canavari & Coderoni*, 2020). This is more pronounced when carbon footprint is calculated from the consumption perspective (*Djekic, Petrovic, Božičković, Djordjevic, & Tomasevic*, 2019). However, the modern consumer's food purchasing decisions depend also on the nutritional quality of food, and the consumer's wellness goals (*Martínez-Ruiz & Gómez-Cantó*, 2016). The current study highlights the importance of incorporating both environmental and nutritional dimensions into carbon footprint calculations. For instance, instead of consuming soft cheeses like ricotta (88.2 kg CO_{2e}/kg protein), individuals who are concerned about both their protein intake and the environment might choose Camembert (32.54 kg CO_{2e}/kg protein) or mozzarella (35.86 kg CO_{2e}/kg protein). For environmentally conscious consumers seeking high calcium content in their diet, Gruyere (1.21 kg CO_{2e}/1000 mg Ca) would be a much better choice than cottage cheese (8.40 kg CO_{2e}/1000 mg Ca).

4. Conclusion

The present study provides better understanding of the environmental impact in relation to nutritional values of cheeses for the purpose of aligning dietary preferences with sustainability goals. Promoting nutritional values as FUs facilitates informed decision-making and encourages environmentally conscious choices, contributing to a more sustainable and responsible approach to food consumption. Finally, the current study intends to combat any type of greenwashing associated with promoting "greener" cheeses by expressing only their carbon footprint per mass.

Future studies could focus on expanding the carbon footprint matrix from the current study by incorporating additional data on plant-based cheese alternatives.

Procena ugljeničnog otiska u proizvodnji sira: Studija o masenim i nutritivnim indikatorima

Ilija Đekić, Nada Šmigić, jelena Miočinović, Zorana Miloradović

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APSTRAKT

Proizvodnja sira obuhvata različite procese pri čemu sama proizvodnja mleka utiče sa preko 85% u ukupnim uticajima na životnu sredinu. Ovo istraživanje je koristilo pojednostavljenu ocenu uticaja na životnu sredinu kako bi procenila ugljenični otisak 13 vrsta sireva u odnosu na utrošak mleka za njihovu proizvodnju (proizvodnja 1 L sirovog kravljeg mleka emituje 1 kg CO2e). Rezultati su prikazani u odnosu na masu sira kao i nutritivne vrednosti (proteini, masti, energija). U odnosu na masu sira, Parmezan je ima najveći ugljenični otisak (16.40 kg CO2e/kg) što je u direktnoj korelaciji sa količinom mleka. Ipak, ako su uzmu nutritivne vrednosti kao funkcionalne jedinice, Rikota je imala najveći ugljenični otisak u odnosu na proteine (88.62 kgCO2e/kg), a švapski sir u odnosu na udeo masti (157.18 kg CO2e/kg) i energetsku vrednosti (1.48 kg CO2e/1000 kJ). Spirmanog koeficijent korelacije za ugljenični otisak je potvrdio korelaciju između nutritivnih vrednosti ($p<0.05$) bez korelacije u odnosu na masu ($p>0.05$). Promovisanje nutritivnih vrednosti kao funkcionalnih jedinica ohrabruje prilagođavanje izbora u ishrani sa ciljevima održivog razvoja.

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