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**MICROBIOLOGICAL SAFETY OF PLANT-BASED AND TRADITIONAL SAUSAGE PRODUCTS: COMPARATIVE ANALYSIS OF SELECTED FOODBORNE PATHOGENS**

S u m m a r y

**Background.** The growing popularity of plant-based alternatives to meat products – driven by health, ethical and environmental concerns – raises questions about their microbiological safety. Differences in raw material composition and production technology may affect the presence of pathogenic microorganisms, however, comparative data in this area remain limited. The aim of the study was to assess the microbiological safety of traditional and plant-based sausages by analyzing the presence and levels of *Salmonella* spp., *Escherichia coli*, *Enterococcus* sp. and *Staphylococcus aureus*. A total of 64 samples were analyzed – 32 meat-based and 32 plant-based products (8 from each of the following categories: boiled sausages, *kabanos* sausages, frankfurters and salami). The samples were tested for the number of *Enterococcus* sp., *S.aureus* and *E.coli*, as well as the presence of *Salmonella* spp. The results were subjected to a statistical analysis using Statistica 13 software.

**Results and conclusions.** *Salmonella* spp. was not detected in any of the samples. *E. coli* was found in 6.2 % of the plant-based products (salami) and in 15.6 % of the meat-based products – specifically in boiled sausages (6.2 %) and salami (9.4 %). The bacterial count in the contaminated samples ranged from  $< 10$  to  $2 \times 10^1$  CFU/g. A similar pattern was observed for *Enterococcus* sp., found in 6.2 % of the plant-based products (salami, up to  $2 \times 10^1$  CFU/g) and in 18.7% of the meat-based ones – frankfurters (6.2 %) and salami (12.5 %). The mean *S. aureus* count was  $1.9 \times 10^1$  CFU/g in plant-based products and  $2.3 \times 10^1$  CFU/g in traditional meat products. A statistical analysis showed no significant differences between the two groups ( $p = 0.8953$ ). The results suggest that plant-based alternatives can be microbiologically comparable to traditional meat products. Nonetheless, ongoing monitoring is recommended, especially in the context of processing and storage conditions, to ensure sustained product safety.

**Keywords:** plant-based meat alternatives, traditional meat-based products, microbial contamination, food safety, plant-based deli meats

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## **Introduction**

In recent years, there has been a significant expansion of the plant-based food market, driven by increasing consumer awareness of health, ethical and religious considerations, as well as environmental concerns [5, 15, 25]. The evolution of consumer perceptions regarding plant-based products over the past decade, particularly in relation to these factors, has been thoroughly analyzed by Runte et al. [21], who reported a systematic increase in the acceptance of and interest in plant-based alternatives to animal-derived foods across three countries. The shift away from meat consumption, or its reduction in favor of plant-based alternatives, has emerged as a leading dietary trend in Poland and other European nations [11, 22]. Consumers are increasingly seeking products perceived as natural, healthy and more sustainable, while maintaining familiar taste profiles and technological forms, such as sausages, frankfurters and other processed meat counterparts. According to the report by the Polish Association of Plant-Based Food Producers [18], the value of the retail market for plant-based products in Poland reached PLN 1.08 billion, with meat, sausage and fish substitutes accounting for PLN 168.3 million. Furthermore, approximately 7 % of Poles report following a plant-based diet, and 40 % are reducing their meat consumption, indicating a steadily growing demand for alternative food options. The "HoReCa Market in Poland – 2025" report also highlights a significant increase in interest in plant-based dishes within the foodservice sector, leading to an expanding range of plant-based products offered by suppliers to professional kitchens [22].

The growing popularity of meat and sausage counterparts is not only due to their perceived superior nutritional value but also their availability and convenience. As reported in the HoReCa Market report [22], consumers consider plant-based alternatives to be healthier, less processed and more natural. However, they are often unaware that such products can also pose microbiological risks - especially when they are ready-to-eat and do not require further thermal processing [4].

To date, research has primarily focused on evaluating the nutritional value, raw material composition and environmental aspects of plant-based foods. Microbiological analyses of these products [11, 19] remain relatively scarce. As demonstrated by Roch et al. [19], the microbiome structure of plant-based products varies depending on raw material composition, production technology and functional additives, which can influence their shelf life and consumer safety. A study conducted in England between 2022 and 2023 revealed that certain vegan substitutes for dairy and meat products may contain potentially pathogenic microorganisms, underscoring the need for monitoring their microbiological quality [24].

Despite the increasing presence of plant-based sausages in retail and foodservice markets, their microbiological safety has not been sufficiently documented. These products, particularly highly processed ones that mimic the structure of traditional

meat, contain plant proteins and technological additives that can create favorable conditions for the growth of microorganisms, including opportunistic and pathogenic species [19, 25]. It is noted that plant-based meat counterparts exhibit high water activity and are neutral to slightly alkaline pH, which - combined with high protein content - promotes microbial spoilage, similar to traditional meat products. Although their physicochemical parameters may resemble those of meat, it remains uncertain whether the level of microbiological risk is comparable, due to different contamination sources and the specific nature of plant-based raw materials [13, 23].

Conversely, meat products have long been recognized as high-risk items concerning microbiological contamination, particularly regarding the presence of bacteria from the genera *Staphylococcus*, *Enterococcus*, *Escherichia* and *Salmonella* [1, 10, 16]. For both product groups, ensuring proper hygiene during production, raw material control and storage conditions is crucial, especially for ready-to-eat products. *Staphylococcus aureus* can be present in both meat and plant-based products, especially those intended for cold consumption, as its presence is often associated with inadequate hygienic conditions during production and packaging [2, 17]. *Escherichia coli*, as an indicator of fecal contamination [16], is a primary parameter for assessing food hygiene, and its presence may result from contamination of raw materials or processing environments. *Enterococcus* spp., although naturally occurring in the environment, can also indicate poor hygienic quality, and their presence in food may suggest inadequate sanitary conditions [10]. The presence of *Salmonella* spp. constitutes an absolute safety criterion in accordance with Regulation (EC) No. 2073/2005 [20].

The inclusion of these microorganisms in the study was based on their role as hygiene and safety indicators, and was not exclusively associated with animal-derived ingredients. Several outbreaks and contamination events have shown that even plant-based foods can harbor these bacteria [3, 4], particularly when hygiene lapses occur during production or distribution. Therefore, analyzing their presence in both plant-based and meat products enables a more complete and objective comparison of microbiological safety between these two food categories.

In light of the above, it is essential to conduct studies comparing the levels of microbiological contamination in plant-based products and their meat counterparts. The aim of this study was to assess the microbiological safety of selected plant-based and traditional sausage products available on the retail market by analyzing the presence and counts of selected pathogenic microorganisms.

## Materials and methods

### Materials

The research material consisted of traditional meat-based sausages (T) (n = 32) and their plant-based counterparts (W) (n = 32), all purchased in the retail market. Four product categories were selected for analysis: boiled sausages, *kabanos* sausages, frankfurters and salami (8 samples from each category). All products were packaged in individual retail units and collected from different production batches. The transport time from purchase to delivery to the laboratory did not exceed one hour. The samples were transported in a thermally insulated container. Products for the analysis were purchased in discount stores and hypermarkets located in the Tri-City area.

### Methods

For each sample, 20 g portions were aseptically collected in a laminar airflow cabinet and homogenized with 180 cm<sup>3</sup> of Ringer's solution using a Stomacher Lab-Blender 400 (Seward, Worthing, United Kingdom). Microbiological analyses were performed using the pour plate or surface plating method, depending on the target microorganism:

- *Enterococcus* spp. were enumerated on D-coccosel agar (bioMérieux, France) and incubated at 37 °C for 48 hours (pour plate method);
- *Staphylococcus aureus* was quantified using Baird-Parker agar supplemented with RPF (bioMérieux, France), incubated at 37 °C for 48 hours (pour plate method);
- *Escherichia coli* was determined on Coli ID selective medium (bioMérieux, France), incubated at 37 °C for 48 hours (pour plate method);
- the presence of *Salmonella* spp. was assessed qualitatively using CHROMagar™ Salmonella Plus (CHROMagar, France), incubated at 37 °C for 18 ÷ 24 hours (surface plating method).

Microbiological analyses were performed using the dilution method, and microorganisms were counted according to PN-EN ISO 7218:2008.

### Statistical analysis

Statistical analyses were performed using Statistica 13 software. The number of *Staphylococcus aureus* was compared between traditional meat-based (T) and plant-based (W) sausage products across different categories. The normality of data distribution within each group and product category was assessed using the Shapiro-Wilk test. A significance level of  $\alpha = 0.05$  was adopted; p-values greater than 0.05 were interpreted as an indication of no significant deviation from normal distribution. For categories in which both groups met the assumption of normality ( $p > 0.05$  in the Shapiro-Wilk test), Student's t-test was applied to compare the mean number of *S. aureus*. In

the cases where the distribution was non-normal ( $p \leq 0.05$ ), the Mann-Whitney U test was used instead.

## Results

No *Salmonella* spp. were detected in any of the analyzed samples, regardless of product type or group. *E. coli* was identified in 6.2 % of the plant-based products, exclusively in salami. In contrast, this bacterium was found in 15.6 % of the meat-based products, including sausages (6.2 %) and salami (9.4 %). The bacterial counts in the positive samples ranged from  $< 10$  to  $2 \times 10^1$  cfu/g, indicating a low but notable level of contamination. A similar pattern was observed for *Enterococcus* sp., detected in 6.2 % of the plant-based samples (salami, with counts not exceeding  $2 \times 10^1$  cfu/g) and in 18.7 % of the meat-based samples. In the latter group, *Enterococcus* sp. was found in frankfurters (6.2 %) and salami (12.5 %), with the highest level reaching  $4 \times 10^1$  cfu/g. The mean count of *S. aureus* was  $1.9 \times 10^1$  cfu/g in plant-based products and  $2.3 \times 10^1$  cfu/g in meat-based products. A statistical analysis revealed no significant difference between the two groups overall ( $p = 0.8953$ ).

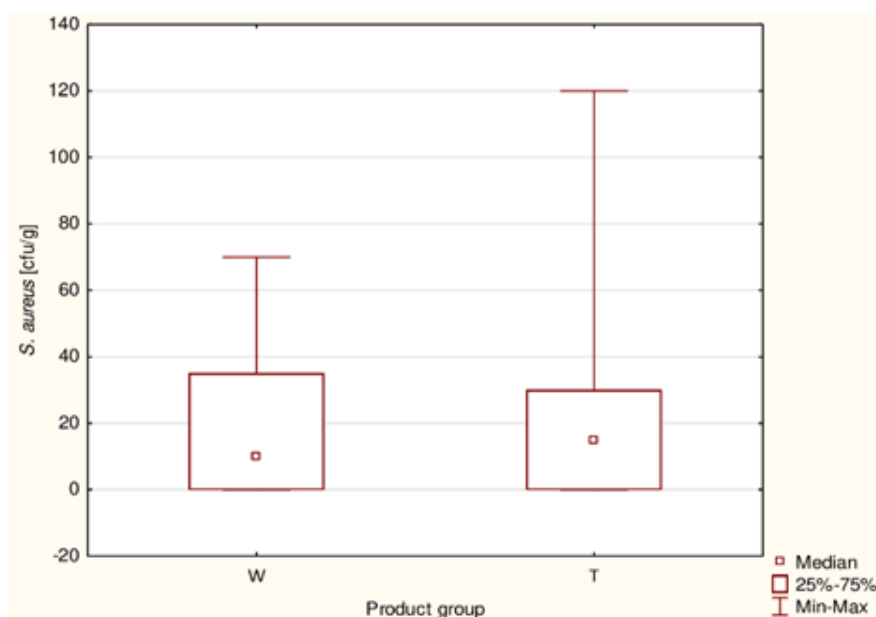


Figure 1. Boxplot showing the distribution of *S. aureus* counts [cfu/g] in plant-based (W) and meat-based (T) sausage products. The square indicates the median, and the whiskers represent the maximum values observed

Rycina 1. Boxplot przedstawiający rozkład liczby *S. aureus* [jtk/g] w kiełbasach roślinnych (W) i mięsnych (T). Kwadrat wskazuje medianę, a wąsy reprezentują maksymalne zaobserwowane wartości

The distribution of *S. aureus* counts in both product groups is illustrated in Figure 1, which shows similar medians and variability, despite a higher maximum value observed in the meat-based group ( $1.2 \times 10^2$  cfu/g vs.  $7 \times 10^1$  cfu/g in plant-based products). However, when the product categories were analyzed separately, significant differences in *S. aureus* counts were observed in salami ( $p = 0.0132$ ) and *kabanos* sausages ( $p = 0.0106$ ), with higher values found in traditional products. No statistically significant differences were noted in the sausage ( $p = 0.6514$ ) and frankfurter ( $p = 0.4037$ ) categories. In addition, the distribution of *S. aureus* counts across concentration ranges was examined (Fig. 2).

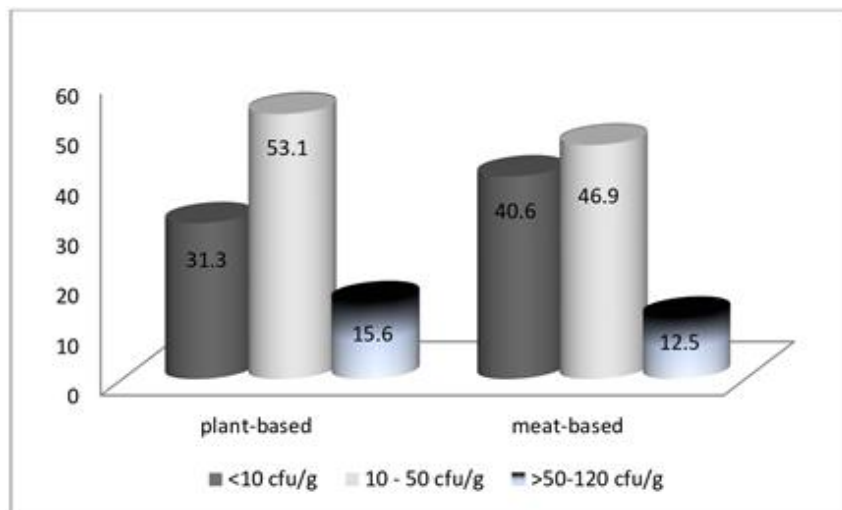


Figure 2. Percentage of samples with a specified *S. aureus* counts [cfu/g] in plant-based and meat-based products

Rycina 2. Procent próbek z określoną liczbą *S. aureus* w produktach roślinnych i mięsnych

The majority of samples in both groups fell within the  $10 \div 50$  cfu/g range – 53.1 % of the plant-based products and 46.9 % of the meat-based ones. Samples with *S. aureus* counts below the detection limit ( $< 10$  cfu/g) accounted for 31.3 % of the plant-based group and 40.6 % of the meat-based group. Higher levels ( $> 50 \div 120$  cfu/g) were less common, observed in 15.6 % of the plant-based products and 12.5 % of the meat-based ones. The highest detected *S. aureus* counts reached  $7 \times 10^1$  cfu/g in plant-based *kabanos* sausages and  $1.2 \times 10^2$  cfu/g in meat-based salami. Figure 3 provides a visual comparison of detection frequencies for the analyzed microorganisms across meat-based and plant-based sausage products.

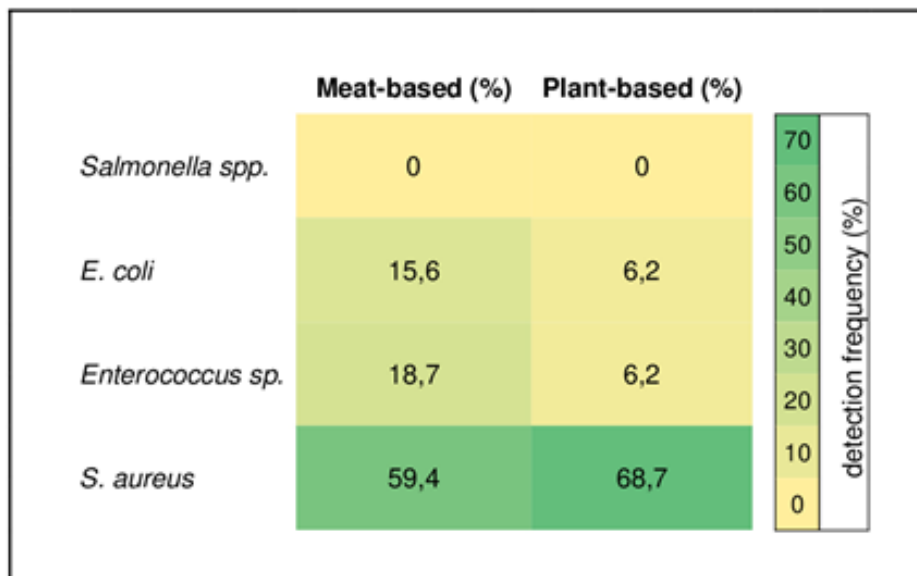


Figure 3. Heatmap showing the detection frequency (%) of selected microorganisms in meat-based and plant-based processed sausage products. Values refer to the percentage of samples in which the respective microorganisms were detected

Rycina 3. Mapa cieplna przedstawiająca częstość wykrycia (%) wybranych mikroorganizmów w wędlinach roślinnych i tradycyjnych. Wartości odnoszą się do odsetka próbek, w których wykryto dany drobnoustrój

## Discussion

It should be emphasized that the technologies applied (e.g. thermal treatment, fermentation, smoking or curing) may significantly influence the microbiological profile of the final product. Consequently, the observed microbial presence results not only from raw material origin but also from the specific production and storage conditions. This constitutes both a limitation and a rationale for further research. The results of the present study indicate that both plant-based and traditional sausage products available on the Polish retail market may be subject to microbiological contamination, although the overall safety profile – especially in relation to *Salmonella* spp. – was satisfactory. The presence of *Salmonella* spp. was not detected in any of the tested samples, which is consistent with the findings of Willis et al. [24] and Barmettler et al. [3], and indicates compliance with the requirements of Regulation (EC) No. 2073/2005 [20]. The presence of *E. coli* was observed more frequently in meat-based products (15.6 %) than in plant-based ones (6.2 %), which confirms earlier observations indicating a greater susceptibility of meat to fecal contamination due to the nature of the raw material and slaughtering processes [16]. Greeson et al. [10] demonstrated that *E. coli*

was present in 72.2 % of tested meat samples, further highlighting the scale of this risk. Although in the present study the level of contamination did not exceed  $2 \times 10^1$  cfu/g, the presence of *E. coli* in ready-to-eat products is undesirable. Certain pathogenic strains of *E. coli* can cause foodborne illnesses associated with toxin production, as well as infections leading to gastrointestinal, renal and central nervous system damage [7]. In the context of plant-based products, research findings are less consistent. Kukulowicz and Kornaga [14] did not detect *E. coli* in the analyzed plant-based meat counterparts. Conversely, Tóth et al. [23] reported the occurrence of *Enterobacteriaceae* (potentially including *E. coli*), exclusively in meat-free products, suggesting that the microbiological risk associated with meat counterparts may be slightly higher than that of meat-containing products. According to the authors, *Enterobacteriaceae* could serve as an indicator of the hygiene level of the production process for these products [23]. Similar findings were reported by Willis et al. [24], who, in a study involving 937 samples of plant-based alternatives to dairy and meat products, found that 5 % of the samples were classified as unsatisfactory in terms of microbiological quality, mainly due to elevated levels of *Enterobacteriaceae* and *E. coli*. The authors emphasized that the presence of these bacteria reflects insufficient hygiene during production, even if the strains detected are not directly pathogenic. Kabisch et al. [13] reported an average *Enterococcus* count of 1.28 log cfu/g ( $1.66 \times 10^2$  cfu/g) in plant-based meat substitutes, whereas in the present study, the mean count of these bacteria in plant-based sausage counterparts was  $< 10$  cfu/g. *Enterococcus* spp. were detected three times more frequently in meat-based products (18.7 %) than in plant-based products (6.2 %). Greeson et al. [10] found that the prevalence of *Enterococcus* in meat samples was 26.2 %, further confirming the significance of this bacterial group as an indicator of hygiene. Although not always pathogenic, *Enterococcus* spp. are considered indicators of poor hygienic practices and potential reservoirs of antibiotic resistance [8]. In the present study, *Enterococcus* spp. were detected in meat-based frankfurters (6.2 %) and salami (12.5 %), as well as in plant-based salami (6.2 %), which is consistent with the observations of Giraffa [9], who reported the presence of enterococci in fermented meat products such as salami and Landjäger at levels ranging from  $10^2$  to  $10^5$  cfu/g. Additionally, *Enterococcus faecium* is capable of surviving heat treatment at 68 °C for 30 minutes, corresponding to the standard processing conditions for frankfurters. Therefore, the reduction of enterococcal contamination in fermented and non-fermented meat products remains a technological challenge, given the survival capabilities of *Enterococcus* spp. during standard processing conditions [9]. *Staphylococcus aureus* was detected in both groups of products, with the mean count being slightly higher in the meat-based samples ( $2.3 \times 10^1$  cfu/g) compared to the plant-based samples ( $1.9 \times 10^1$  cfu/g). These findings are consistent with the observations of Kabisch et al. [13], who reported a similar mean *S. aureus* count ( $2.4 \times 10^1$  cfu/g) in plant-based products availa-

ble at the retail level in Germany, although the maximum level in their study reached  $6.5 \times 10^2$  cfu/g. Barmettler et al. [3] detected *S. aureus* in 2 % of samples, with bacterial counts reaching around  $5.5 \times 10^2$  cfu/g. In the present study, the maximum count of *S. aureus* in the plant-based products was  $7 \times 10^1$  cfu/g, which is in line with earlier results obtained by Kukułowicz and Kornaga [14]. Statistically significant differences in *S. aureus* counts were observed for two product categories – salami and *kabanos* sausages – suggesting that the factors such as raw material composition, casing quality and technological conditions, including processing temperature, may influence the risk of contamination with this pathogen [6, 17]. The mean *S. aureus* count in the meat products was lower than the levels reported by Hassanina et al. [12] for sausages ( $6 \times 10^3$  cfu/g). Moreover, the maximum *S. aureus* count recorded by Hassanina et al. ( $1.3 \times 10^4$  cfu/g) was approximately two logarithmic cycles higher than the maximum values obtained in the present study [12]. The presence of *S. aureus* in ready-to-eat products raises particular concern due to the pathogen's ability to produce enterotoxins; therefore, its occurrence in such foods should be treated with special caution [17]. Despite the common perception of plant-based foods as being microbiologically safer, studies indicate that these products may present their own specific risks. Tóth et al. [23] and Roch et al. [19] emphasized that highly processed plant-based meat alternatives, due to their high water activity ( $a_w > 0.95$ ), neutral pH and high protein content, can promote the growth of spoilage microbiota, despite the absence of animal tissues. Moreover, potential sources of contamination may include plant-based raw materials, secondary contamination or insufficient hygiene of production lines. The overall microbiological quality of both traditional and plant-based sausages in this study was satisfactory. Nevertheless, the results highlight the need for continuous monitoring of the microbiological safety of both product types, particularly in view of their growing popularity on the market. As noted by Barmettler et al. [3], even products declared as vegan or “natural” may exhibit unexpected levels of contamination, underscoring the importance of conducting individual risk assessments for different categories of food.

## Conclusion

1. Both traditional and plant-based sausage products analyzed in this study generally complied with microbiological safety standards, particularly regarding the absence of *Salmonella* spp.
2. However, the presence of *S. aureus*, *E. coli* and *Enterococcus* spp. in the selected samples indicates the need for continuous microbiological surveillance, especially in ready-to-eat products.
3. Significant differences in *S. aureus* counts between salami and *kabanos* sausages suggest that product composition and processing parameters may influence contamination risk. Maintaining strict hygiene controls during production and storage

is essential to ensure the safety of both conventional and plant-based meat alternatives.

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## BEZPIECZEŃSTWO MIKROBIOLOGICZNE ROŚLINNYCH I TRADYCYJNYCH WYROBÓW WĘDLINIARSKICH: ANALIZA PORÓWNAWCZA WYBRANYCH PATOGENÓW PRZENOSZONYCH PRZEZ ŻYWNOSĆ

### Streszczenie

**Wprowadzenie.** Rosnąca popularność roślinnych alternatyw dla produktów mięsnych – napędzana względami zdrowotnymi, etycznymi i środowiskowymi – rodzi pytania dotyczące ich bezpieczeństwa mikrobiologicznego. Różnice w składzie surowcowym oraz technologii produkcji mogą wpływać na obecność drobnoustrojów patogennych, jednak dane porównawcze w tym zakresie pozostają ograniczone. Celem badania była ocena bezpieczeństwa mikrobiologicznego tradycyjnych i roślinnych wędlin poprzez analizę obecności i liczebności *Salmonella* spp., *Escherichia coli*, *Enterococcus* sp. oraz *Staphylococcus aureus*. Analizie poddano łącznie 64 próbki – 32 produkty mięsne i 32 produkty roślinne (po 8 z każdej kategorii: parówki, kabanosy, frankfurterki oraz salami). W próbkach oznaczano liczbę *Enterococcus* sp., *S. aureus*, *E. coli* oraz obecność *Salmonella* spp. Uzyskane wyniki poddano analizie statystycznej z wykorzystaniem programu Statistica 13.

**Wyniki i wnioski.** Obecność *Salmonella* spp. nie została stwierdzona w żadnej z badanych próbek. *E. coli* wykryto w 6,2 % produktów roślinnych (salami) oraz w 15,6 % produktów mięsnych – w parówkach (6,2 %) i salami (9,4 %). Liczebność bakterii w skażonych próbkach mieściła się w zakresie od  $<10$  do  $2 \times 10^1$  jtk/g. Podobny wzorzec zaobserwowano dla *Enterococcus* sp., wykrytych w 6,2 % produktów roślinnych (salami, maksymalnie  $2 \times 10^1$  jtk/g) oraz w 18,7 % produktów mięsnych – frankfurterkach (6,2 %) i salami (12,5 %). Średnia liczba *S. aureus* wyniosła  $1,9 \times 10^1$  jtk/g w produktach roślinnych oraz  $2,3 \times 10^1$  jtk/g w tradycyjnych wyrobach mięsnych. Analiza statystyczna nie wykazała istotnych różnic pomiędzy obiema grupami ( $p = 0,8953$ ). Uzyskane wyniki sugerują, że roślinne alternatywy mogą być mikrobiologicznie porównywalne z tradycyjnymi

produktami mięsnymi. Niemniej jednak zaleca się stałe monitorowanie ich bezpieczeństwa, zwłaszcza w kontekście procesów produkcji i przechowywania.

**Słowa kluczowe:** roślinne alternatywy mięsa, tradycyjne produkty mięsne, zanieczyszczenie mikrobiologiczne, bezpieczeństwo żywności, roślinne wyroby wędliniarskie ☒