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RESEARCH ARTICLE

Microbiological status of vegan ground meat products from German retail

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Abstract

The microbiological status of 10 raw plant-based ground meat products was assessed to obtain insight into contamination levels and the types of bacteria present in these meat analogues. The total bacterial counts at the end of the best before date varied greatly from below 1.0 $\log_{10}$ CFU/g to 8.31 $\log_{10}$ CFU/g, while the median count was 3.89 $\log_{10}$ CFU/g. For each product, the lactic acid bacterial counts were similar, although generally between ca. 0.5 and 1 $\log_{10}$ lower than the total bacterial counts, indicating that lactic acid bacteria were a majority in the microbiota of these products. While the median counts of toxigenic pathogens were generally very low (< 1.0 $\log_{10}$ CFU/g), the maximum counts detected in some samples could reach up to ca. 3.0 $\log_{10}$ CFU/g for presumptive *Staphylococcus aureus* and *Bacillus cereus.* No *Listeria monocytogenes* colonies were obtained in this investigation; however, other *Listeria* spp. were detected. Thus, the results show that a (re)contamination of these products by pathogenic bacteria can be a potential safety concern. Furthermore, the detection of presumptive *B. cereus* and the isolation of various *Clostridium* species from these products indicates that spore-formers may have survived the food processing and therefore, could pose a safety concern, which should be assessed in further studies.

Keywords Plant-based meat · Ground meat substitutes · Meat analogues · Pathogens · Food safety

1 Introduction

The increased consumption of plant-based products concomitant with a decrease in meat consumption is an ongoing consumer trend that is based on health, ethical and ecological motivations. Especially, the trend towards consumption of vegan and vegetarian meat analogues that are similar to meat products in their taste and consistency is growing. In Europe, the meat consumption is 69 kg/per capita/year on average (OECD et al. 2019). In Germany, the per capita consumption of meat decreased from > 60 kg/year before 2019 to 52 kg/year in 2022 (Bundesinformationszentrum Landwirtschaft 2023; Statista 2023). At the same time, sales of vegan and vegetarian meat analogues reached 458.2 million Euro in 2021, showing an increase of 62.2% within the years 2019–2021 (Statistisches Bundesamt 2022).

Meat analogues are produced from plant-based protein raw materials, amongst which products made from soy, wheat or pea currently dominate. The proteins are extracted from plants as isolated protein, protein concentrate, or protein texture. Plant protein ingredients for meat analogues production include flours (10–20% protein), concentrates (55–60% protein), isolates (> 80% protein), and texturized protein (50–70% protein) (Akharume et al. 2021; Toth et al. 2021). Based on their constituents and structure, as well as type of processing used in production, meat analogues products can be grouped into 3 different categories:

(i) emulsion products, similar to emulsion-type sausages such as e.g., Frankfurter or Mortadella,
(ii) ground meat or products of this and,
(iii) products which mimic complete meat pieces such as chicken or steak meat (Kyriakopoulou et al. 2021).

The production of meat alternatives relies on a complex interaction of raw materials, food additives and/or processing aids, as well as structure-forming processes such as extrusion. Common elements during processing of meat analogues are the necessity for a heat processing step in order

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to obtain the desired structure, and an additional thermal step to decontaminate the final product for microbiological safety. While such a heating step will undoubtably contribute towards the safety and shelf life, an encompassing and long history of the microbiological safety and quality of such products is, based on their relatively short market availability, still lacking. Also, it should be noted that if protective cultures are added to plant-based products to increase their safety, a final heat decontamination step would not be applied to prevent the inactivation of the protective culture.

As plant-based meat analogues are high in protein and water activity ($a_W > 0.95$) with an only weakly acidic pH (pH > 5.5), they are highly permissive for microbial spoilage (Wild et al. 2014; He et al. 2020). Although these parameters are similar to meat, it is still questionable whether their food safety risks are comparable, because of the contamination potential (exposure) and plant sources. Plant-based meat can carry pathogenic bacteria originating from the raw ingredients. Although most of these would be inactivated by heating during the extrusion process, some endospore-forming bacteria, such as *Clostridium* (*Cl.*) spp. or *Bacillus* (*B.*) spp., could possibly survive (Wild et al. 2014). Furthermore, products may become re-contaminated, and one study showed that several bacterial contaminants occurred on plant-based meats from retail, including *Latilactobacillus sakei*, *Enterococcus faecium* and *Carnobacterium divergens* (Geeraerts et al. 2020). In general, plant-based meats have been less investigated with regard to food safety aspects.

The aim of this study was to determine the microbiological status of raw vegan ground meat at the retail level and thus to obtain insight into the levels of contamination and the types of bacterial contaminants associated with these products. Furthermore, we aimed to determine physicochemical characteristics such as pH and water activity of these products.

2 Materials and methods

2.1 Plant-based ground meat products

Ten vegan ground meat substitutes (Table 1) produced by different companies made from soy (n = 5), pea (n = 4) or oats/wheat (n = 1) were obtained in 2021 from local supermarkets and discounters in the area of Kiel, northern Germany. All products were produced from conventional cultivated protein sources and were packaged in a modified atmosphere (except product 10, which was frozen). No information on the package indicated which gasses were used for modified atmosphere packaging. Eight were labelled that the product should be kept at a maximum of 7 °C, while one was recommended for storage at maximum of 4 °C and one for storage at − 18 °C, respectively. All sampled products were obtained on average 9.5 (± 5.6 days) prior to expiry of the best before or use by dates, except the frozen product with 9 months before the best before date. Four products were labelled with a best before date, while six products were labelled with a use by date (Table 1). All products contained instructions for a proper heating. Microbiological analysis of products was done in technical triplicates using products that stemmed from different production batches, and plate counts were performed in duplicate.

Table 1 Investigated plant-based ground meat analogue products and selected characteristics and physicochemical properties

Product no.	Main protein source	Best before or use by date	Max. storage temperature (°C)	Additives added	Mean[a] pH ± SD	Mean[a] a_W ± SD
1	Oats	Best before	7	None declared	6.27 ± 0.097	0.978 ± 0.004
2	Pea	Best before	7	Stabilizer: methylcellulose	6.39 ± 0.036	0.971 ± 0.002
3	Soy	Use by	7	Iron diphosphate; Vit. B12	5.97 ± 0.067	0.970 ± 0.003
4	Soy	Use by	4	Acidity regulators: potassium lactate, potassium acetate; stabilizer: methylcellulose; protective culture	6.22 ± 0.156	0.96 ± 0.004
5	Soy	Use by	7	Stabilizer: methylcellulose	6.05 ± 0.040	0.983 ± 0.004
6	Soy	Use by	7	Acidity regulators: potassium lactate, sodium acetate; thickening agent: methylcellulose; preservative: potassium sorbate	6.06 ± 0.287	0.971 ± 0.005
7	Pea	Use by	7	Thickening agents: carboxymethylcellulose, cellulose	6.56 ± 0.122	0.969 ± 0.005
8	Soy	Best before	7	Thickening agent: methylcellulose; protective culture	5.83 ± 0.180	0.98 ± 0.005
9	Pea	Use by	7	Thickening agent: methylcellulose; food colorant: Lycopin	5.40 ± 0.057	0.971 ± 0.002
10	Pea	Best before	− 18	Stabilizer: methylcellulose; antioxidant: rosemary extract	6.46 ± 0.079	0.983 ± 0,004

[a]Mean value from triplicate product determinations

2.1.1 Determination of pH and water activity

For determination of pH, 25 g of food product were weighed into a stomacher bag and 250 ml of quarter-strength Ringer's solution were added. The sample was placed in a stomacher (Stomacher 400 Circulator, Seward, Germany) for 150 s at 230 rpm. The pH was determined using a pH probe and an Education Line pH meter (Mettler Toledo, Germany). The water activity (a_W) was measured by placing a raw vegan ground meat substitute sample in a sterile Petri dish using a Hygrolab a_W-meter (Rotronic, Germany).

2.1.2 Microbiological analyses

For microbiological analyses, 25 g of food product sample were weighed into a stomacher bag, 225 ml of quarter-strength Ringer's solution were added and the sample was stomached at 230 rpm for 150 s. The samples were further diluted in a tenfold dilution series and 0.1 ml volumes of appropriate dilutions were spread onto microbiological agar growth media in duplicate. Where appropriate, 0.1 ml or 1 ml of sample after stomaching were pour plated with 20 ml of the appropriate microbiological agar growth medium cooled to 50 °C after autoclaving. For microbiological analysis, the aerobic mesophilic bacterial counts were assessed on plate count agar (VWR International, Darmstadt, Germany) for 72 h at 30 °C. Enterobacteria were counted on violet red bile dextrose (VRBD) (VWR International) agar after 24 h aerobic incubation at 37 °C. Enterococcal counts were determined on kanamycin-esculin azide (KEAA) (Merck, Darmstadt, Germany) agar after incubation at 42 °C for 24 h, while lactic acid bacterial (LAB) counts were assessed on de Man, Rogosa and Sharpe (VWR International) agar incubating at 25 °C for 48 h. Listeria were counted on Listeria agar according to Ottavani & Agosti (ALOA) (VWR International) which was incubated for 48 h at 37 °C, while numbers of coagulase-positive *Staphylococcus aureus* (black and convex colonies of 1–2 mm size, surrounded by a clear zone) were determined on Baird Parker (BP) (VWR International) agar after 48 h at 37 °C.

Spore-forming bacteria were isolated using an initial heating step to inactivate vegetative cells and to activate spore germination. A qualitative assessment of the presence of sulfite-reducing clostridia (*Clostridium* spp.) was performed in differential reinforced clostridial medium (DRCM) broth (VWR International). For this, 1 ml of the vegan ground meat sample that had been homogenized 1:10 in quarter-strength Ringer's solution was mixed with 9 ml of DRCM medium in a test tube, overlayered with ca. 4 cm layer sterile mineral oil and then heated for 10 min in a water bath at 75 °C. Following the heating step, the tube was incubated for 72 h at 30 °C and observed for gas formation as well as black discoloration of the medium, indicating growth of sulfite-reducing clostridia. Presence of *B. cereus* was also shown to occur by using 10 ml after homogenizing the food sample in quarter-strength Ringer's solution and heating this at 80 °C for 10 min. Where appropriate, the sample was diluted in a tenfold dilution series and 0.1 ml volumes were plated out onto polymyxin egg yolk mannitol bromothymol blue (PEMBA) agar (Sifin Diagnostics, Berlin, Germany) and plates were incubated for 24 h at 37 °C and when needed a further 24 h at room temperature.

In general, plates with 10–300 colonies per plate were counted for quantitative analysis. In the lowest dilution steps, colony counts < 10 were also counted if the duplicates showed consistent results and the colony morphologies could be clearly assigned or the identity could be confirmed by further differentiation.

2.1.3 DNA isolation, 16S rRNA gene PCR and sequencing

DNA was isolated from all presumptive *Clostridium* isolates obtained from DRCM broth after streaking for purity on Columbia blood agar (Oxoid, Wesel, Germany) and incubating for 48 h at 30 °C anaerobically. DNA was isolated using the ZR Fungal/Bacterial DNA Miniprep kit (Zymo Research) according to the manufacturer's instructions. For 16S rRNA gene PCR, the primers 27For (5′–3′) and 1492Rev (5′–3′) were used (0.1 mM final concentration) in a reaction containing 2.5 µl template DNA, 8 µl distilled PCR grade water and 12.5 µl 2 × dream Taq PCR Master mix, and the PCR was performed in 35 cycles (95 °C for 30 s, 53 °C for 30 s, 72 °C for 1.5 min, preceded by 5 min at 95 °C initial denaturation and followed by 72 °C final elongation for 10 min). The DNA was sequenced commercially at MWG Biotech (Ebersberg, Germany). The sequences were analyzed using Geneious (v9.0.5; Biomatters Limited, New Zealand). After alignment and trimming the sequence was checked for homology to other sequences in the 16S rRNA RefSeq databank using the BLASTn algorithm.

2.1.4 Statistical and visual analysis

All colony counts were $\log_{10}$-transformed. For statistical analyses and figures, the values below the limit of detection (LOD) were substituted by the half of the LOD value. For example, values below the LOD of < 1 $\log_{10}$ CFU/g ($\triangleq$ < 10 CFU/g) were arbitrarily set at a value of 0.70 $\log_{10}$ CFU/g ($\triangleq$ 5 CFU/g). Statistical analysis and graphical presentation of the data were performed using Excel software (Office Professional Plus 2019, Microsoft, Redmond, USA) and JMP (v17, SAS Software, Cary, USA).

3 Results

In this study, the mean pH values of vegan ground meat analogues ranged from pH 5.40 to pH 6.56 (Table 1), with a mean pH of 6.13 ± 0.34 and a median pH of 6.16 and therefore, were weakly acidic (Fig. 1a). Although product 4 and product 8 claimed to have been produced with protective culture, they did not have a lower pH than all other products and were also only weakly acidic at pH 6.22 ± 0.156 (product 4) and 5.83 ± 0.180 (product 8) (Table 1). Two of the products (i.e., product 4 and 6) were labelled to contain the acidity regulators potassium lactate, as well as either potassium acetate (product 4, pH 6.22 ± 0.156) or sodium acetate (product 6, pH 6.06 ± 0.287). The mean water activity values were typical of high moisture foods with a_W of > 0.85 (Erkmen und Bozoglu 2016), ranging from 0.960 to 0.983 (Table 1) with a mean of 0.974 ± 0.0079 and a median of 0.974 (Fig. 1b). The pH or a_w values were not obviously different for the plant protein sources from pea or soy, as these values were distributed over the range of values determined in the study for these plant proteins (Fig. 1a, b). The oat/wheat product included in this study showed pH and a_w values above the median value for all the products investigated in this study. However, as only one oat/wheat product was investigated it cannot be concluded that this is a general characteristic for these specific plant protein products.

The mesophilic total aerobic plate counts varied greatly, ranging from 0.70 to 8.32 $\log_{10}$ CFU/g, with a median count of 3.90 $\log_{10}$ CFU/g and a mean count of 4.33 ± 1.95 $\log_{10}$ CFU/g (Fig. 2; Table 2). The LAB clearly constituted the majority of the total mesophilic bacteria present in these products, as these bacteria were also detected in high numbers, ranging from 0.70 to 7.98 $\log_{10}$ CFU/g, with a median count of 3.30 $\log_{10}$ CFU/g and a mean count of 3.75 ± 2.11 $\log_{10}$ CFU/g (Fig. 2; Table 2). The fact that the LAB counts resembled the total aerobic plate counts in both ranges, median and mean, but were slightly lower than these, indicated that LAB constituted the majority of the bacteria determined on the total plate counts. However, as the total aerobic plate count was generally higher than the LAB count, this also indicated that other bacteria than LAB occurred to some extent. Furthermore, the mean total aerobic plate counts and mean LAB counts were very high at 8.08 ± 0.22 $\log_{10}$ CFU/g and 7.91 ± 0.08 $\log_{10}$ CFU/g, respectively, for product 8, which typically resemble the counts for fermented products to which LAB starter cultures are added. Product 8 was claimed to contain protective culture; as the very high mean total aerobic plate count was thus very similar to the mean LAB count, this indicated that the protective LAB culture predominated the microbial composition of the product.

Low levels of enterobacteria, enterococci, presumptive *B. cereus*, *Listeria* spp. and *Staphylococcus aureus* were detected, i.e. at median counts that were below to near the detection limit of 0.7 to 1.0 $\log_{10}$ CFU/g. While the mean counts ranged from 0.98 ± 0.48 $\log_{10}$ CFU/g for *Enterobacteriaceae* to 1.39 ± 0.87 $\log_{10}$ CFU/g for presumptive *B. cereus* and 1.39 ± 0.84 $\log_{10}$ CFU/g for *S. aureus* (Fig. 2; Table 2), the numbers of *Listeria* spp. isolated on ALOA medium were 1.12 ± 0.73 $\log_{10}$ CFU/g. None of the samples, however, showed *L. monocytogenes* typical colonies on the agar. Enterococci could be detected with a mean of 1.28 ± 0.73 $\log_{10}$ CFU/g (Table 2).

The maximum numbers of potential toxigenic pathogens determined in this study were the highest for presumptive *B. cereus*, with a maximum value of 3.15 $\log_{10}$ CFU/g which was determined for the frozen product 10 (Table 2). Interestingly, frozen product 10 showed a similar total aerobic plate count of 3.66 ± 0.22 $\log_{10}$ CFU/g, indicating that most

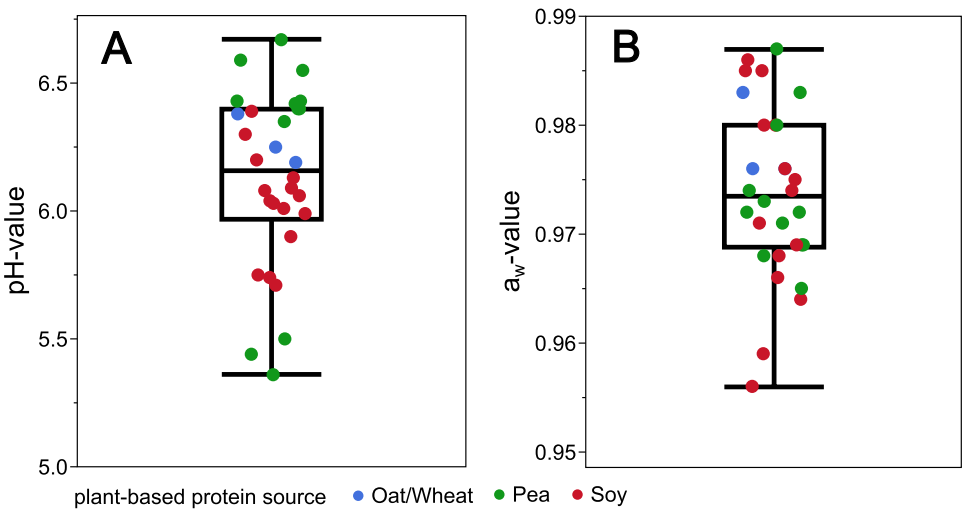

Fig. 1 Boxplot for values of pH (**A**) and a_w (**B**) from ground meat analogues samples obtained from the German retail market. Colors indicate the plant-based protein source

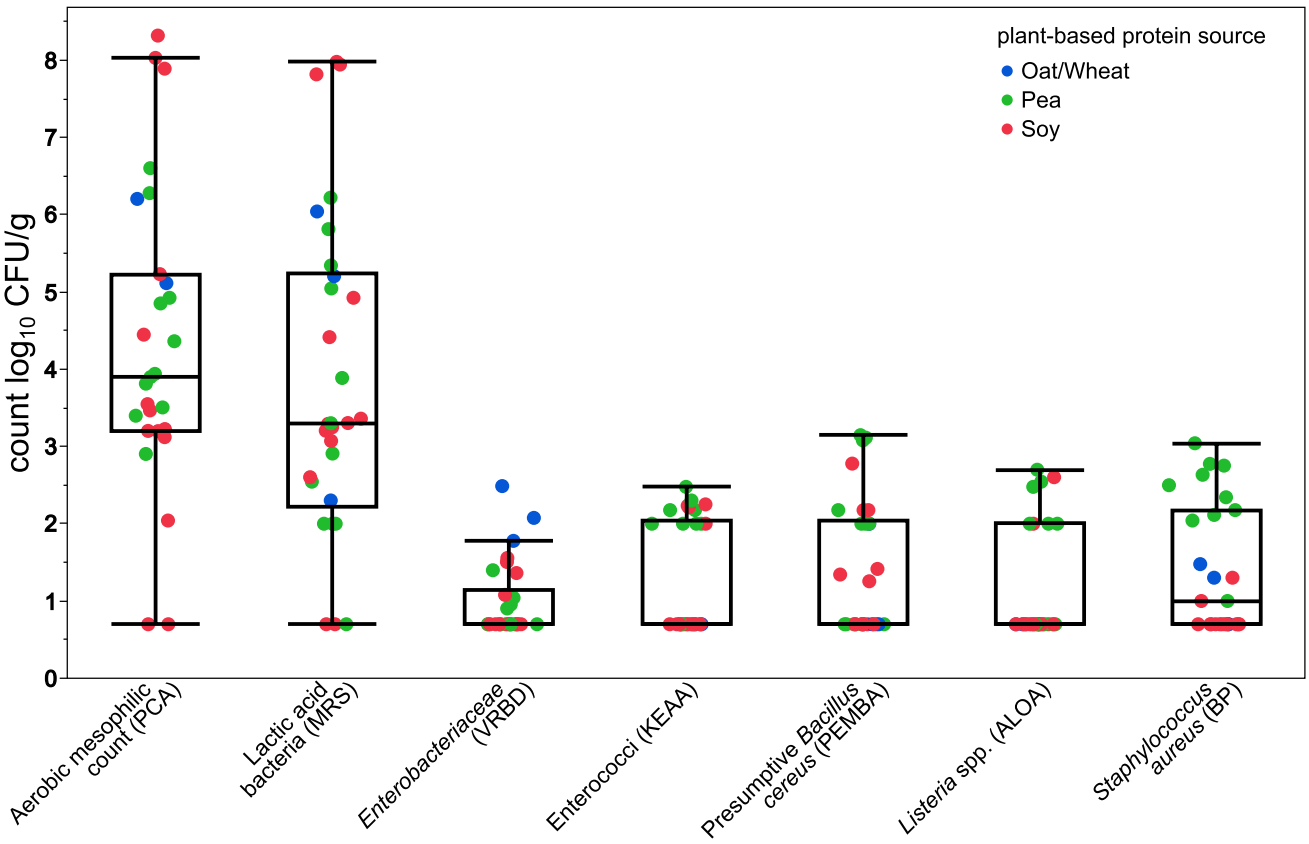

Fig. 2 Box plots showing bacterial counts (log_{10} CFU/g) of bacteria associated with raw plant-based ground meat analogues from the German retail market. Colors indicate the plant-based protein source. (a) Total aerobic, mesophilic count. (b) Lactic acid bacterial count. (c) Enterobacterial count. (d) Enterococcal count. (e) Presumptive *B. cereus* count. (f) *Listeria* spp. Count. (g) *S. aureus* count

Table 2 Mean bacterial cell counts (log_{10} CFU/g) of plant-based ground meat analogue products. Each product was investigated in triplicates

	Mean bacterial cell count in log_{10} CFU/g ± Standard Deviation						
	Aerobic mesophilic count (PCA)	Lactic acid bacteria (MRS)	*Enterobacteriaceae* (VRBD)	Enterococci (KEAA)	Presumptive *Bacillus cereus* (PEMBA)	*Listeria* spp. (ALOA)	*Staphylococcus aureus* (BP)
All products	4.33 ± 1.95	3.75 ± 2.11	0.98 ± 0.48	1.28 ± 0.73	1.39 ± 0.87	1.12 ± 0.73	1.39 ± 0.84
Product 1	5.66 ± 0.77	4.52 ± 1.96	2.11 ± 0.36	0.70 ± 0	1.13 ± 0.75	0.70 ± 0	1.16 ± 0.41
Product 2	1.15 ± 0.78	0.70 ± 0	0.70 ± 0	0.70 ± 0	0.70 ± 0	0.70 ± 0	0.70 ± 0
Product 3	3.32 ± 0.2	3.21 ± 0.12	1.47 ± 0.1	2.23 ± 0.02	1.34 ± 0.08	0.70 ± 0	0.70 ± 0
Product 4	4.84 ± 0.55	3.98 ± 1.22	0.70 ± 0	1.13 ± 0.75	2.38 ± 0.35	1.77 ± 0.97	1.15 ± 0.21
Product 5	3.27 ± 0.18	3.29 ± 0.08	0.70 ± 0	0.70 ± 0	0.70 ± 0	0.70 ± 0	0.70 ± 0
Product 6	5.02 ± 1.18	5.69 ± 0.6	0.70 ± 0	2.16 ± 0.15	0.70 ± 0	1.13 ± 0.75	2.08 ± 0.05
Product 7	5.30 ± 1.17	3.85 ± 1.31	1.05 ± 0.35	0.70 ± 0	1.19 ± 0.85	1.13 ± 0.75	1.67 ± 0.95
Product 8	8.08 ± 0.22	7.91 ± 0.08	0.83 ± 0.22	0.70 ± 0	0.70 ± 0	0.70 ± 0	0.70 ± 0
Product 9	3.40 ± 0.5	2.81 ± 0.97	0.70 ± 0	1.57 ± 0.75	2.00 ± 0	1.13 ± 0.75	2.48 ± 0.3
Product 10	3.66 ± 0.22	1.57 ± 0.75	0.85 ± 0.14	2.22 ± 0.24	3.11 ± 0.03	2.57 ± 0.11	2.81 ± 0.21

of the bacteria that made up the total aerobic plate count were presumably *B. cereus*. Assuming that the product was immediately frozen after production and a heating step was employed during production, this may imply that spores of these bacteria possibly survived the heating step. Clearly it would be important to assess this more closely in future

investigations. In this study, the maximum *Listeriae* and presumptive *S. aureus* counts were 2.70 $\log_{10}$ CFU/g and 3.04 $\log_{10}$CFU/g, respectively. This indicated that these probably stemmed from recontamination and that recontamination of such products with pathogenic bacteria, similar to the case of meat products, cannot be excluded.

Clostridia were detected in 7 out of the investigated 10 products (70%). While in products 5 and 7 the 16S sequence did not resolve in a species-level identification (genus *Clostridium*), the rest of the isolated clostridial strains could be identified by this method. Thus, for product 1, the *Clostridium* isolated from 1 of the technical triplicate samples could be identified as *Cl. cochlearium* (99.3% homology), while in product 3 a *Cl. putrefaciens* (99.3% homology) strain could be isolated from one of the triplicate samples. For product 4, clostridia could be isolated from all three of the triplicate samples. Thus, *Cl. sporogenes* was isolated from 2 (99.2% homology, both) and 1 *Cl. thiosulfatireducens* (99.8% homology) from the third replicate. The latter also produced a black sediment in DRCM medium. In product 8, *Cl. fallax* could be isolated from two replicate samples (99.5 and 99.5% homology, respectively) while in product 9 a *Cl. sporogenes* (99.6% homology) strain could be isolated from 1 of the replicate samples and a *Cl. sulfidigenes* (99.5% homology) strain from another.

Regarding the total aerobic and lactic acid bacterial counts, as well as for *Enterobacteriaceae*, presumptive *Bacillus*, *Listeria* spp. and *S. aureus* counts, it could be observed that these were distributed along the complete range of counts determined in the study for both the soy and the pea-based products. Thus, the pea or soy proteins fractions respectively were not exclusively adulterated with a specific group of microorganisms or specific bacteria. No such conclusion could be drawn for products based on oat/wheat as only 1 product was investigated in the study.

4 Discussion and conclusion

So far, there are only few studies which have focused on the microbiological quality and safety of meat analogues, despite the relatively huge growth of these products on the market the last few years. Plant-based meat analogues are classified differently from meat products according to the EU regulation (EC) 2073/2005. Also, the guideline and warning values of the German Association for Hygiene and Microbiology [Deutsche Gesellschaft für Hygiene und Mikrobiologie (DGHM)] for meat products do not apply to these products. Therefore, the microbiological parameters defining the quality for these products are so far relatively unknown and reliable safety data are still lacking. It is clear, however, that the characteristics of meat analogue products include high water activity, high amounts of nutrients and only a weakly acidic pH (Table 1; Fig. 1), which renders these products highly susceptible to microbial spoilage by a wide range of microorganisms that can be selected for solely by the storage conditions of these products.

Yadav et al. (2015) showed that the total bacterial counts of (vegan) meat roll analogues increased from ca. 2.9 $\log_{10}$ CFU/g to 4.6 $\log_{10}$ CFU/g over 12 days storage at 4 °C. Geeraerts et al. (2020) investigated vegetarian and vegan meat analogues products such as Bologna sausage, ham and salami-like products. In their study, a total of 8 and 6 sliced vegetarian and vegan products, respectively, were bought at Belgian supermarkets and kept at 7 °C until their expiration date. The total microbial counts varied greatly. While most of the vegetarian products showed only low total bacterial numbers of < 2 $\log_{10}$ CFU/g, a few products showed total bacterial numbers of ca. 3 $\log_{10}$ CFU/g. For the vegan products, two also displayed low total counts of ca. 2 $\log_{10}$ CFU/g, while one had a total count of ca. 4 $\log_{10}$ CFU/g and two further products had very high total plate counts of ca. 8 $\log_{10}$ CFU/g (Geeraerts et al. 2020). Interestingly, the LAB counts closely resembled the total counts in most cases. The majority of bacteria isolated from the vegetarian products were identified as *Latilactobacillus* (*Lb.*) *sakei*, whereas the majority of bacteria from vegan products were identified as either *Lb. sakei*, *Enterococcus faecium* or *Carnobacterium divergens*. Duthoo et al. (2022) investigated the microbiota of a curry-flavored vegetarian sliced product imitating chicken charcuterie through the shelf life using a 16S based metagenomics approach. This study showed that at the end of the best before date, *Lb. sakei* dominated the microbiota followed by other LAB such as *Streptococcus* and *Weissella*. They also showed that the total aerobic and anaerobic plate counts and LAB counts at the end of the shelf-life period deviated between 4.1 and 5.5 $\log_{10}$ CFU/g and were not significantly different from each other at each examined sampling moment. Sulphite-reducing clostridia, *Enterobacterales*, *B. cereus*, *Brochothrix thermosphacta* and *Enterococcus* spp. on the other hand, were rarely countable (Duthoo et al. 2022), but could be isolated. The vegan meat substitute products in the studies of Geeraerts et al. (2020) and of Duthoo et al. (2022) thus showed the expected characteristic of LAB dominance in products packages under modified atmosphere packaging (MAP) conditions, which is a common feature also of MAP meat products. The products in this study were also kept under modified atmosphere packaging and it was, therefore, not surprising that the LAB counts resembled the total aerobic plate counts and LAB were therefore probably also the major spoilage bacteria present in these products. LAB such as *Weissella*, carnobacteria, enterobacteria and *Lb. sakei*, as identified in the studies of Geeraerts et al (2020) and Duthoo et al. (2022), are major spoilage bacteria of meats stored at low temperatures. *Lb. sakei* can be problematic if these bacteria reach high

numbers, as they can produce an unwanted acid taste or contribute to ropey slime production in products where sucrose may be present (Geeraerts et al. 2020). Carnobacteria also are known to spoil meats, however, these do not grow well at storage temperatures below 12°C, as they are less cold-adapted than *Lb. sakei* (Duthoo et al. 2022). Enterococci were also found in some products in our study on kanamycin esculin azide agar (Table 2). *E. faecium* was determined to be one of the most prevalent microorganisms in vegetarian and vegan meat analogue products in the study of Geeraerts et al. (2020) and also occurred in the study by Duthoo et al (2022). The presence of these bacteria may raise concerns regarding their potential to harbor virulence factors, their involvement in infections as well as their potential to harbor transferable antibiotic resistance genes (Franz et al. 2011; Werner et al. 2013; Geeraerts et al. 2020).

Plant-based meat analogues are mainly produced through thermoplastic extrusion (Osen et al. 2014; Pietsch et al. 2017; Hadi und Brightwell 2021) which involves a heating step. A concern, therefore, is that spore-forming bacteria (which may include pathogenic species such as *B. cereus* or *Cl. perfringens*) from the raw materials survive the heating during the extrusion process and germinate in the extruded product. Plant-based meats can carry spore-forming bacteria originating from the raw ingredients. In our study we were able to isolate various *Clostridium* species in 7 of the 10 products. In a research project (LikeMeat) funded by the European Union, *Cl. sporogenes* ATCC 19404 spores used to inoculate protein ingredients were rendered inactive (below detection level) by extrusion, even though *B. amyloliquefaciens* (AB255669 and LMA008A < 1000 CFU/g) were detected in the final extrudates, possibly due to re-contamination post-extrusion (Wild 2014). This study suggested that the extrusion process can render spores inactive, yet the probability of product becoming re-contaminated was also confirmed by occurrence of other bacteria such as *Enterococcus durans, Exigobacterium acetylicum, Acinetobacter* spp. and *Staphylococcus* spp. in the extruded and uninoculated samples (Wild 2014). Furthermore, a remarkable study of Pernu et al. (2020) determined the incidence of *Cl. botulinum* in vegetarian sausages and could show the presence of the botulinum toxin gene occurring in 32% of the products. The authors thus speculated that spore germination and growth of these bacteria could occur in such products, in case of non-compliance with the cold chain.

A study of vegetarian products on local markets in central Taiwan showed the presence of *S. aureus* in 18.1% of 320 samples taken from the market. The microbial loads varied, but numbers of *Bacillus* spp. reached up to ca. 4 $\log_{10}$ CFU/ml and numbers of *B. cereus* up to 3 $\log_{10}$ CFU/g (Fang et al. 1999). Such high *Bacillus* numbers were similar as determined for product 10 in this study (Table 2). Wild et al. (2014) speculated that although endospore-forming bacteria such as *Bacillus* and *Clostridium* spp. should be inactivated to a high extent during the extrusion process, some of these bacteria may potentially survive.

It is still unclear at what processing level microorganisms enter this product group. The involvement of pre-products, additives such as spices, or the production environment has not been sufficiently investigated to understand the entry pathways. Vegan ground meat analogues are not intended to be eaten raw. The information to heat the product thoroughly was declared on all products tested. However, it is not easy for the consumer to judge whether they have been sufficiently heated (core temperature of 70 °C for at least 2 min). Unlike meat, the color of these products does not change during heating. Therefore, an optical perception by color change is not possible.

In conclusion, therefore, the results of our study confirmed the observation of Geeraerts et al. (2020) who found that plant-based meat analogues generally have lower microbial loads than their meat equivalents. Plant-based meat analogues are not sterile products and refrigerated storage is essential to limit microbial growth. Furthermore, in our study, as in the study of Geeraerts et al. (2020) and Duthoo et al. (2022), LAB appeared to be the major spoilage bacteria. Although these bacteria would be more problematic from a spoilage than a safety point of view, as they may potentially cause souring, gas production in packages or ropey slime, the enterococci subset of LAB may actually also constitute a food safety concern. The presence of other *Listeria* spp. was noted, although we did not isolate *Listeria monocytogenes* from the products. Furthermore, we also found presumptive *B. cereus* and *S. aureus* at a maximum count level of up to 3 $\log_{10}$ CFU/g. These cell counts are generally considered insufficient for toxin production that could endanger consumers, especially since the products are not consumed raw. The presence of such *Listeria* and *S. aureus,* however, point towards the potential for recontamination and thus the need for adequate processing hygiene. More worrisome are also the consistent findings of the presence of endospore-forming bacteria in these products, sometimes at elevated levels. The origin of these bacteria is probably from plant materials, and their presence suggests that either the spores have survived the extrusion process or the plant-based meat analogues has become re-contaminated after extrusion processing. Further research should, therefore, investigate the inactivation of relevant endospore-forming pathogens during the extrusion process and the germination and growth characteristics of these bacteria in plant-based meat analogue products under typical packaging and storage conditions.

The limitations of this study are the small number of samples of vegan ground meat and that in addition, some products showed a relatively wide range of total bacterial counts.

This could be an indication that the product hygiene is not consistent as is known for other product types and shows that manufacturers may be faced with problems of varying quality of raw materials. Furthermore, this may be a result of the fact that the market for plant-based alternatives is highly variable and that a great variety of these products are manufactured. Thus, our results serve as an initial evaluation on the microbiological quality, which should be investigated more in-depth over a longer time period with a wide range of samples in order to generate a sufficient scientific database for plant-based meat analogues.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Akharume FU, Aluko RE, Adedeji AA (2021) Modification of plant proteins for improved functionality. Compr Rev Food Sci F 20:198–224. https://doi.org/10.1111/1541-4337.12688

Bundesinformationszentrum Landwirtschaft (2023) Versorgung mit Fleisch in Deutschland im Kalenderjahr 2022 (vorläufig). Hg. v. Bundesanstalt für Landwirtschaft und Ernährung (BLE). Online verfügbar unter https://www.ble.de/DE/BZL/Daten-Berichte/Fleisch/fleisch_node.html (**Accessed 23 June 2023**)

Duthoo E, Kde R, Leroy F, Weckx S, Heyndrickx M, Rasschaert G (2022) To culture or not to culture: careful assessment of metabarcoding data is necessary when evaluating the microbiota of a modified-atmosphere-packaged vegetarian meat alternative throughout its shelf-life period. BMC Microbiol. https://doi.org/10.1186/s12866-022-02446-9

Erkmen O, Bozoglu TF (2016) Food microbiology. Principles into practice. Wiley, Chichester

Fang TJ, Chen CY, Kuo WY (1999) Microbiological quality and incidence of *Staphylococcus aureus* and *Bacillus cereus* in vegetarian food products. Food Microbiol 16(4):385–391. https://doi.org/10.1006/fmic.1998.0256

Franz CMAP, Huch M, Abriouel H, Holzapfel W, Gálvez A (2011) Enterococci as probiotics and their implications in food safety. Int J Food Microbiol 151(2):125–140. https://doi.org/10.1016/j.ijfoodmicro.2011.08.014

Geeraerts W, Vuyst Lde, Leroy F (2020) Ready-to-eat meat alternatives, a study of their associated bacterial communities. Food Biosci 37. https://doi.org/10.1016/j.fbio.2020.100681

Hadi J, Brightwell G (2021) Safety of alternative proteins technological, environmental and regulatory aspects of cultured meat, plant-based meat insect protein and single-cell protein. Foods. https://doi.org/10.3390/foods10061226

He J, Evans NM, Liu HZ, Shao SQ (2020) A review of research on plant-based meat alternatives: driving forces, history, manufacturing, and consumer attitudes. Compr Rev Food Sci F 19(5):2639–2656. https://doi.org/10.1111/1541-4337.12610

Kyriakopoulou K, Keppler JK, van der Goot AJ (2021) Functionality of ingredients and additives in plant-based meat analogues. Foods. https://doi.org/10.3390/foods10030600

OECD; Food; Agriculture Organization of the United Nations (2019) OECD-FAO Agricultural Outlook 2019–2028

Osen R, Toelstede S, Wild F, Eisner P, Schweiggert-Weisz U (2014) High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. J Food Eng 127:67–74. https://doi.org/10.1016/j.jfoodeng.2013.11.023

Pernu N, Keto-Timonen R, Lindström M, Korkeala H (2020) High prevalence of *Clostridium botulinum* in vegetarian sausages. Food Microbiol 91:103512. https://doi.org/10.1016/j.fm.2020.103512

Pietsch VL, Emin MA, Schuchmann HP (2017) Process conditions influencing wheat gluten polymerization during high moisture extrusion of meat analog products. J Food Eng 198:28–35. https://doi.org/10.1016/j.jfoodeng.2016.10.027

Statista (2023) Fleischkonsum in Deutschland pro Kopf bis 2022 | Statista. Hg. v. ble.de. Online verfügbar unter https://de.statista.com/statistik/daten/studie/36573/umfrage/pro-kopf-verbrauch-von-fleisch-in-deutschland-seit-2000/ (**Accessed 23 June 2023**)

Statistisches Bundesamt (2022) Fleischersatz weiter im Trend: Produktion stieg 2021 um 17% gegenüber dem Vorjahr. Online verfügbar unter https://www.destatis.de/DE/Presse/Pressemitteilungen/2022/05/PD22_N025_42.html (**Accessed 23 June 2023**)

Toth AJ, Dunay A, Battay M, Illes CB, Bittsanszky A, Suth M (2021) Microbial spoilage of plant-based meat analogues. Appl Sci-Basel. https://doi.org/10.3390/app11188309

Werner G, Coque TM, Franz CMAP, Grohmann E, Hegstad K, Jensen L et al (2013) Antibiotic resistant enterococci-Tales of a drug resistance gene trafficker. Int J Med Microbiol 303(6–7):360–379. https://doi.org/10.1016/j.ijmm.2013.03.001

Wild F, Czerny M, Janssen AM, Kole APW, Zunabovic M, Domig KJ (2014) The evolution of a plant-based alternative to meat from niche markets to widely accepted meat alternatives. Agro Food Ind Hi Tec 25(1):45–49

Wild F (2014) Final Report Summary - LIKEMEAT (High quality meat-like products - from niche markets to widely accepted meat alternatives). https://cordis.europa.eu/project/id/262560/reporting

Yadav P, Ahlawat SS, Jairath G, Rani M, Bishnoi S (2015) Studies in chemical properties and shelf life of developed chicken meat analogue rolls. Haryana Veterinarian 54(1):25–28