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Growth and survival of common spoilage and pathogenic bacteria in ground beef and plant-based meat analogues

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ABSTRACT

To better understand the microbial quality and safety of plant-based meat analogues, this study investigated the changes of native microflora present in soy- and pea-based meat analogues (SBM and PBM) and compared them with ground beef (GB). SBM, PBM, and GB were also artificially inoculated with meat spoilage microorganisms, *Pseudomonas fluorescens* and *Brochothrix thermosphacta*, and pathogenic microorganisms, *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*; the fitness of these bacteria was evaluated during storage at refrigerated and/or abused temperatures. Results showed that the initial total aerobic plate count (APC), coliform, lactic acid bacteria (LAB), and mold/yeast (M/Y) counts for GB could be as high as 5.44, 2.90, 4.61, and 3.45 log CFU/g, while the highest initial APC, coliform, LAB, and M/Y counts found in SBM were 3.10, 2.00, 2.04, and 1.95 log CFU/g, and were 3.82, 2.51, 3.61, and 1.44 log CFU/g for PBM. The batch-to-batch differences in microbial counts were more significant in GB than in SBM and PBM. Despite the different initial concentrations, there was no difference among APC and LAB counts between the three meat types by the end of the 10-day 4 °C storage period, all approaching ca. 7.00 log CFU/g. Artificially-inoculated *B. thermosphacta* increased by 0.76, 1.58, and 0.96 log CFU/g in GB, PBM, and SBM respectively by the end of the refrigeration storage; *P. fluorescens* increased by 4.92, 3.00, and 0.40 log CFU/g in GB, PBM, and SBM respectively. Under refrigerated storage conditions, pathogenic bacteria did not change in GB and SBM. *L. monocytogenes* increased by 0.74 log in PBM during the 7-day storage at 4 °C. All three pathogens grew at abused storage temperatures, regardless of the meat type. Results indicated that plant-based meat could support the survival and even growth of spoilage and pathogenic microorganisms. Preventive controls are needed for ensuring the microbial quality and safety of plant-based meat analogues.

1. Introduction

Meat and meat products are important components in human diets because of their high nutritional value. Unfortunately, meat production and consumption are facing challenges, such as ecological impacts, public health issues, and ethical concerns (Boukid et al., 2021; Tóth et al., 2021). Made from vegetable ingredients, plant-based meat analogues are effective meat alternatives that have similar appearance, texture, and flavor with animal-origin meat but avoid the above-mentioned public concerns (Bakhsh et al., 2021; Boukid et al., 2021).

Traditional meat and meat products are susceptible to microbiological contamination because of the rearing and production environments for animals, as well as the products themselves being suitable environments for the growth and survival of spoilage and pathogenic bacteria. The growth of microorganisms and oxidation, as well as enzymatic autolysis, are three major mechanisms responsible for meat spoilage (Iulietto et al., 2015). Microorganisms often responsible for meat spoilage include *Pseudomonas* spp., *Enterobacteriaceae*, lactic acid bacteria (LAB), *Flavobacterium*, and *Brochothrix thermosphacta*, depending on the storage temperature and packaging conditions (Casaburi et al., 2015; Doulgeraki et al., 2012; Pennacchia et al., 2011). For example, *Pseudomonas* spp., especially *Pseudomonas fluorescens,* is usually predominant in refrigerated meat given its high proteolytical activity and tolerance to low temperatures (Bahlinger et al., 2021; Doulgeraki et al., 2012). LAB species that are most involved in meat spoilage include heterofermentative lactobacilli, leuconostocs, *Carnobacterium* spp., homofermentative *Lactobacillus* spp., and *Pediococcus* spp. (Hu et al., 2009; Iulietto et al., 2015). The overgrowth of LAB leads to the production of lactic acid, CO_2 gas, ethanol, acetic acid, botanic acid, and acetoin, leading to defects such as off-odor and ropy slime (Iulietto et al.,

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2015; Kröckel, 2013). Lactic acid bacteria and *B. thermosphacta* have been major concerns in spoiled meats under anaerobic conditions (Bahlinger et al., 2021; Doulgeraki et al., 2012).

The microbial safety of meat products has also been a public concern for many years. Foodborne pathogens like *Salmonella* spp., Shiga-toxin-producing *Escherichia coli*, and *Listeria monocytogenes* have been identified from meat and meat products (Sofos and Geornaras, 2010; Xu et al., 2019). Omer et al. (2018) summarized foodborne outbreaks related to meat and meat products during the period from 1980 to 2015 and found that most of these outbreaks were caused by *Salmonella* and pathogenic *E. coli*. Although a variety of *Salmonella* serotypes cause human diseases, most meat-related pathogenic *E. coli* outbreaks were attributed to *E. coli* O157:H7 (Omer et al., 2018). With the development of meat processing industries, *L. monocytogenes* has become much more of a concern because of its widespread environment, strong ability in adhering to processing equipment, and easy transferring from processing plants to meat surfaces (Chaitiemwong et al., 2014; Sofos and Geornaras, 2010).

Although the growth of spoilage bacteria and pathogens has been studied in traditional meat systems, information about whether these organisms can grow and become a threat to the quality and safety of plant-based meat analogues remains limited (Luchansky et al., 2020). According to a recent report (November 2022), one plant-based meat processing facility has experienced mold and bacteria contamination due to the unsanitary condition, indicating the potential microbial safety and quality challenges associated with plant-based meat analogues (Demetrakakes, 2022). Meat analogues are usually made of plant-based ingredients such as plant proteins, vegetal lipids, polysaccharides, and some flavoring and coloring agents (Boukid et al., 2021). Special procedures such as texturization and extrusion are also needed in the production of meat analogues to form a meat-like texture (Boukid et al., 2021). Thus, the background microorganisms in meat analogues could be quite different those in animal-origin meat. Besides, meat analogues provide a relatively different nutrition environment, pH, and inner structure for microorganisms to adhere, invade, and proliferate, which may influence the survival and growth of both spoilage and pathogenic bacteria (Hadi and Brightwell, 2021; Luchansky et al., 2020; Xiang et al., 2017).

Therefore, the goal of this study is to fill our knowledge gaps associated with meat analogues by evaluating and comparing the microbial quality and safety of ground beef with commercially available meat analogues (soy- and pea-based meat). The specific aims are 1) investigating the levels and changes of native microorganisms present in meat analogues during storage at refrigerated and abused temperatures, and 2) exploring the survival and/or growth potential of common meat spoilage bacteria (*P. fluorescens and B. thermosphacta*) and foodborne pathogens (*Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes*) in plant-based meat analogues during refrigeration and/or under temperature-abused conditions and comparing that with ground beef.

2. Materials and methods

2.1. Meat and meat analogue samples

Raw vacuum packaged ground beef (GB), soy-based meat (SBM), and pea-based meat (PBM) were purchased at local grocery stores in Davis, California. The GB used in this study contains 85 % lean and 15 % fat. According to ingredient labels, SBM is primarily composed of soy protein concentrate, coconut oil, and sunflower oil. PBM is primarily composed of pea protein isolate, expeller-pressed canola, and refined coconut oil. Once purchased, all samples were stored at −20 °C in original packaging until usage. To thaw, the frozen meat products were kept in their original packaging and placed in a cold-water bath with the cold water with ice (ca. 0 °C) being replaced every 30 min (USDA, 2016).

2.2. Bacterial cultures

Brochothrix thermosphacta and rifampicin-resistant pathogenic strains used in this study were kindly provided by Dr. Azlin Mustapha at the University of Missouri and Dr. Linda J. Harris at the University of California, Davis. Both *Brochothrix thermosphacta* and *Pseudomonas fluorescens* were induced to rifampin resistance (100 μg/ml) by using the gradient plate method (Smith et al., 1982). Rifampicin-resistant pathogenic strains used in this study were *S.* Enteritidis PT30 (LJH 636, an almond-outbreak strain), *S.* Gaminara (LJH 1220, an orange-juice-outbreak strain), *S.* Tennessee (LJH 1244, a peanut-butter-outbreak strain), *S.* Montevideo (LJH 1245, a pistachio isolate), *S.* Saintpaul (LJH 1375, a walnut isolate), *E. coli* O157:H7 strain LJH 1153 (a lettuce-outbreak isolate), LJH 1213 (an apple-juice-outbreak isolate), LJH 1214 (a cantaloupe-outbreak isolate), LJH 1216 (a spinach-outbreak isolate), LJH 1378 (a cookie-dough isolate), *L. monocytogenes* LJH 1222 (serotype 4b 1/2c, a raw-cabbage-outbreak isolate), LJH 1223 (serotype 4b, a milk-outbreak isolate), LJH 1224 (1/2a, a milk-outbreak isolate), LJH 1225 (4b, a beef-outbreak isolate), and LJH 1229 (a tomato isolate). A five-strain cocktail of each pathogen genera was prepared for inoculation. All strains were maintained at − 80 °C in tryptic soy broth (TSB; Becton, Dickinson and Company (BD), Sparks, MD) supplemented with 15 % glycerol before use.

2.3. Preparation of inocula

The frozen stock culture of each strain was streaked onto tryptic soy agar (TSA, BD) supplemented with 100 μg/ml of rifampicin (TSAR; Biosynth International, Itasca, IL). Plates were then incubated at 25 °C for 24 h. A well-isolated colony was picked from each plate and then transferred into 10 ml of tryptic soy broth (TSB) supplemented with rifampin at 100 μg/ml (TSBR) and incubated at 37 °C or 25 °C for 24 h. On the next day, one 10-μl loopful of the overnight culture was pipetted into 10 ml of fresh TSBR and incubated at 37 °C or 25 °C for another 24 h. The overnight cultures were washed three times by centrifugation with 1 × phosphate buffered saline (PBS, pH 7.4) and resuspended in 10 ml of PBS. The culture was then diluted to each of the desired cell concentrations of ca. 6.00 log CFU/ml.

For pathogenic bacteria, the preparation of the five-strain cocktail followed Liu et al. (2021) and Liu et al. (2022) with modifications. To prepare the cocktails, overnight broth culture was spread onto TSAR plates (250 μl/plate) and incubated at 37 °C for 24 h. Following the incubation, 5 ml of 1 × PBS was pipetted onto each plate, and the cell lawn was scraped with an L-shaped spreader (Cole-Parmer, Swedesboro, NJ, US). re-suspended cells in 1 × PBS were pipetted into a 15-ml Falcon™ tube (Jackson Dickinson and Company, Franklin Lakes, NJ). The cocktail of each strain was prepared by combining equal volumes (1 ml) of every strain and mixing well. Serial dilutions were made in 1 × PBS to achieve target inoculum levels of ca. 6.00 log CFU/ml.

2.4. Inoculation of meat and meat analogues

The prepared inoculum was inoculated into the meat or meat analogue samples at a 1:1,000 wt ratio to reach a final concentration of ca. 3.00 log CFU/g. The original packaging was opened aseptically, and the prepared inoculum of pathogenic or spoilage bacteria was applied in drops evenly on the thawed meat products by using a pipette. After inoculation, each sample (454, 453, and 340 g of GB, SBM, and PBM, respectively) was transferred to a sterile one-gallon Ziploc bag (SC JOHNSON, Racine, WI) and massaged by hand for 2 min to achieve a homogenized distribution of bacteria in meat or meat analogues. Uninoculated meat products were transferred to sterile Ziploc bags after the package was opened and stored at respective storage temperatures for the monitoring of background microorganisms.

2.5. Storage conditions and sampling points

The behavior of native and artificially-inoculated spoilage microorganisms in meat and meat analogues was monitored in a refrigerator (4 °C) for 10 days, mimicking the home storage time and condition. Subsamples were taken on Days 0, 3, 5, and 10. On these sampling days, the total aerobic bacteria (APC), lactic acid bacteria (LAB), yeast and mold (Y/M), and coliforms of the uninoculated meat and meat analogues as well as the behavior of the inoculated *P. fluorescens*, and *B. thermosphacta* were monitored. The off-odor situation of meat samples at each sampling points was recorded with the following criteria: no odor, slight off-odor, moderate off-odor, and strong off-odor (Pohlman et al., 2002). Besides, the color and overall appearance of the samples were evaluated and recorded.

To monitor the behavior of pathogenic bacteria, meat and meat analogue samples spiked with rifampin-resistant *E. coli* O157: H7, *L. monocytogenes*, or *Salmonella* cocktails were stored at 4 °C for 7 days and at temperature-abused conditions for 24 h. The abused temperatures tested included ambient temperature (ca. 22 °C) and 32 °C (USDA, 2016). Subsamples were taken on Days 0, 1, 3, and 7 when being stored at 4 °C and at hours 0, 2, 6, and 24 when being stored at 22 °C and 32 °C for enumeration. For the samples stored at 22 or 32 °C, changes of the native APC, LAB, Y/M counts, and coliform counts in uninoculated meat and meat analogues were also monitored.

2.6. Measurement of the meat and meat analogue pH during storage

Uninoculated samples stored under the above conditions were used for pH measurement during storage. At each sampling point, three 10-gram uninoculated meat or meat analogue samples were taken and each was mixed with 40 ml of Milli Q water to achieve 1:5 dilution (USDA, 1998). The mixtures were then homogenized using the Smasher (Smasher^{TM}, BioMérieux Industry, Hazelwood, MO) for 1 min. The pH of the homogenate was measured by using a pH meter (FiveEasay pH Meter F20, Mettler Toledo, Schwerzenbach Switzerland) following the manufacturer's instruction.

2.7. Microbiological analysis

All samples were examined for the presence of background *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* before use following the Food and Drug Administration's Bacteriological Analytical Manual (Andrews et al., 2018; Feng et al., 2020; Hitchins et al., 2017). For the analyses of native microorganisms, three 10-gram subsamples were taken from each storage condition at every sampling point for each type of meat or meat analogue. Each subsample was combined with 90 ml of 1 × PBS and homogenized in the stomacher for 1 min. The homogenized samples were serially diluted in 1x PBS, and dilutions were plated onto the following media (100 μl of each dilution/per plate; two plates of each agar for every dilution): (i) Plate Count agar (PCA, BD) for APC; (ii) CHROMagar ECC (ECC, DRG International) for coliform; (iii) De Man, Rogosa and Sharpe agar (MRS, BD) for LAB; and (iv) Dichloran Rose-Bengal Chloramphenicol agar (DRBC, BD) for total Y/M counts.

To enumerate the surviving artificially-inoculated pathogenic or spoilage bacteria, homogenized inoculated meat and meat analogue samples were serially diluted in 1 × PBS and plated onto TSAR and selective agar plates. The selective agar used was Xylose Lysine Tergitol 4 agar (XLT-4, BD) for *Salmonella*, MacConkey agar (MAC, BD) for *E. coli* O157:H7, Modified Oxford agar (MOX, BD) for *L. monocytogenes*, Streptomycin Thallous Acetate Actidione agar (STAA, Oxoid) for *B. thermosphacta,* and Cetrimide Fusidin Cephaloridine agar (CFCA, BD) for *P. fluorescens*. To inhibit the background microorganisms, 100 μg/ml of rifampicin was added to selective agar plates as needed. PCA, ECC, XLT-4, MAC, MOX, and TSAR plates were incubated at 37 °C for up to 48 h. MRS plates were incubated at 37 °C for 24 h in anaerobic conditions, and the counts on MRS plates were confirmed after 48 h of incubation. DRBC plates were incubated at room temperature for 5 days in dark, and STAA and CFCA plates were incubated at 25 °C for 48 h. The limit of detection (LOD) by direct plating was 2.0 log CFU/g of inoculated or uninoculated meats.

Table 1
Ph changes of the ground beef (GB), soy-based meat analogs (SBM), and pea-based meat analogs (PBM) during the 7-day refrigeration storage at 4 °C and 24-hour storage at 22 and 32 °C.

		pH		
Storage temperatures	**Sampling points**	**GB**	**SBM**	**PBM**
4 °C	Day 0	5.89 ± 0.05^{Aa}	6.16 ± 0.06^{Ba}	7.38 ± 0.17^{Ca}
	Day 3	5.64 ± 0.04^{Ab}	6.23 ± 0.02^{Ba}	7.00 ± 0.02^{Cb}
	Day 5	5.63 ± 0.02^{Ab}	6.22 ± 0.02^{Ba}	6.91 ± 0.01^{Cb}
	Day 10	5.27 ± 0.12^{Ac}	6.22 ± 0.03^{Ba}	6.26 ± 0.11^{Bc}
22 °C	0 h	5.89 ± 0.05^{Aa}	6.16 ± 0.06^{Ba}	7.38 ± 0.17^{Ca}
	2 h	5.76 ± 0.02^{Aa}	6.13 ± 0.02^{Ba}	7.29 ± 0.01^{Ca}
	6 h	5.80 ± 0.03^{Aa}	6.12 ± 0.02^{Ba}	7.42 ± 0.03^{Ca}
	24 h	5.44 ± 0.02^{Ab}	6.13 ± 0.04^{Ba}	7.45 ± 0.01^{Ca}
32 °C	0 h	5.89 ± 0.05^{Aa}	6.16 ± 0.06^{Ba}	7.38 ± 0.17^{Ca}
	2 h	5.74 ± 0.04^{Aa}	6.09 ± 0.02^{Ba}	7.38 ± 0.08^{Ca}
	6 h	5.78 ± 0.05^{Aa}	6.12 ± 0.04^{Ba}	7.52 ± 0.05^{Ca}
	24 h	5.52 ± 0.06^{Ab}	5.18 ± 0.01^{Bb}	5.58 ± 0.10^{Ab}

*Values are means ± standard deviations, n = 6. Different uppercase letters represent significant differences of pH values at the same sampling points among three meat types. Different lowercase letters represent significant differences of pH for each type of meat among four sampling timepoints ($P < 0.05$).

2.8. Statistical analysis

Two independent trials were conducted for each experiment, with three samples taken at every sampling point for each trial (n = 6). Analysis of variance (ANOVA) and Tukey's multiple comparison test were performed using Prism (Version 9.0) to analyze the differences in pH values; bacterial populations among groups, including different meat types; various sampling points during storage at three storage temperatures; and the use of different enumeration agar. Differences between means were considered significant when *P*-values were<0.05.

2.9. Predictive models for describing pathogen behaviors in meat and meat analogues

Counts obtained from the nonselective agar for three pathogens during the storage at three temperatures were used to fit the primary linear regression model. Combase, available at https://www.combase.cc/index.php/en/), was used to estimate growth rates. The primary linear regression model is described as below:

$$N = a.t + b$$

Where N is the number of viable cells (log CFU/g), a is the proportionality constant (growth rate) between time and the number of viable cells (log CFU/g hour), b is the initial population (log CFU/g).

Based on growth rate of primary models and corresponding storage temperatures, the secondary linear regression model was constructed by using Prism (Version 9.0):

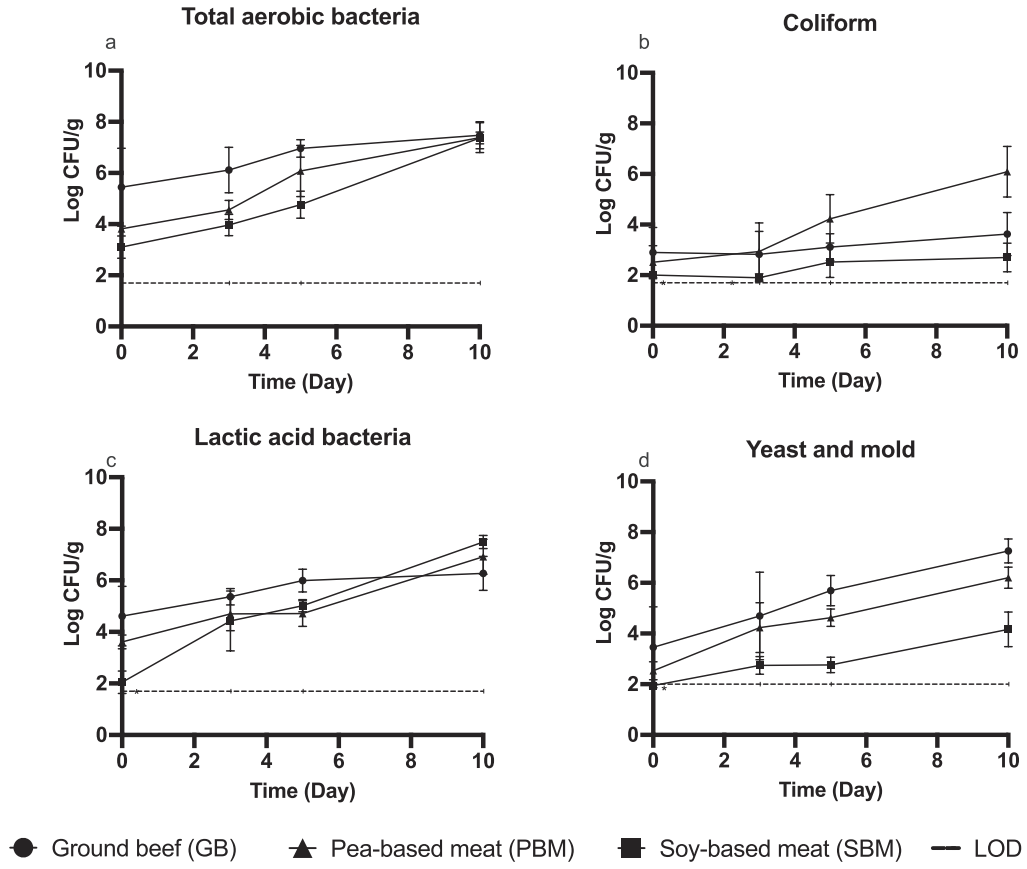

Fig. 1. Changes of the native microflora present in ground beef and plant-based meats during the 10-day storage at 4 °C. (a) total aerobic bacteria, (b) coliform, (c) lactic acid bacteria, and (d) yeast and mold counts. *represents that the microbial counts of meat samples at that sampling point were below limit of enumeration (LOD = 2 Log CFU/g).

$$a_T = k.T + c$$

Where a_T is the proportionality constant (growth rate) between time and the number of viable cells (log CFU/g hour) at specific storage temperatures, T is the storage temperature (°C), k is the proportionality constant between the growth rate and storage temperature (log CFU/g hour °C), and c is the inferred initial growth rate at 0 °C (log CFU/g). The validation of the secondary model was performed based on calculated mean error and root mean square error of prediction (RMSEP) following methods by Skjerdal et al. (2021).

3. Results

3.1. pH changes of meat and meat analogues during storage

The initial pH of GB, SBM, PBM was 5.89 ± 0.05, 6.16 ± 0.06, and 7.38 ± 0.17 respectively. After 10 days of storage at 4 °C, the pH of GB decreased to 5.27 ± 0.12 and the pH of PBM dropped from 7.38 ± 0.17 to 6.26 ± 0.11 ($P < 0.05$). The pH of SBM didn't change significantly during storage, with the final pH being 6.22 ± 0.03 on Day 10 (Table 1). When stored at the ambient temperature (22 °C) for 24 h, the pH of GB decreased to 5.44 ± 0.02 ($P < 0.05$) while the pH of SBM and PBM didn't change significantly. Significant pH drops were observed from all samples when they were kept at 32 ℃ for 24 h ($P < 0.05$), with the final pH of GB, SBM, and PBM decreased to 5.52 ± 0.12, 5.18 ± 0.01, and 5.58 ± 0.10, respectively. Among the three meat types, the degree of pH change in PMB was higher than in GB and SBM at 4 °C and 32 °C.

3.2. Changes of indigenous microflora in meat and meat analogues during storage

Fig. 1 presents the changes of indigenous microflora present in GB, SBM, and PBM during the 10-day storage at 4 ℃. GB had the highest initial native microflora among the three types of meats, followed by PBM and SBM. The initial APC, coliform, lactic acid bacterial, and total Y/M counts were 5.44 ± 1.52, 2.90 ± 0.98, 4.61 ± 1.16 and 3.46 ± 1.60 log CFU/g respectively for GB. Although the APC counts for the three types of meats were significantly different on Day 0, their final population APC counts were all approximately 7.41 log CFU/g (Fig. 1**a**). By the end of the 10 days of refrigeration storage, PBM had the highest coliform levels (6.09 ± 1.00 log CFU/g) followed by GB (3.62 ± 0.86 log CFU/g) and SBM (2.70 ± 0.57 log CFU/g) (Fig. 1**b**). The initial LAB counts for GB, SBM, and PBM were 4.61 ± 1.15, 2.04 ± 0.43, and 3.61 ± 0.27 log CFU/g respectively. During storage, the growth of LAB was more significant in SBM and PBM than in GB. Approximately 2.65 and 3.88 log increases of LAB were observed for SBM and PBM respectively by the end of the storage (Fig. 1**c**). GB had approximately 5.50 log CFU/g of Y/M counts on Day 0, significantly higher than SBM and PBM. At the end of storage, the mean Y/M populations were 7.25 ± 0.47, 4.17 ± 0.69, and 6.20 ± 0.42 log CFU/g for GB, SBM, and PBM respectively (Fig. 1**d**).

Changes in the indigenous microflora in GB and plant-based meats during storage at abused temperatures are shown in Figs. 2 and 3. The use of different batches of meat samples led to the differences seen in the initial background microflora counts. As shown in Figs. 2 and 3, the initial APC counts for GB, SBM, and PBM used for the temperature-abuse storage studies were 2.36 ± 0.19, below the limit of enumeration, and

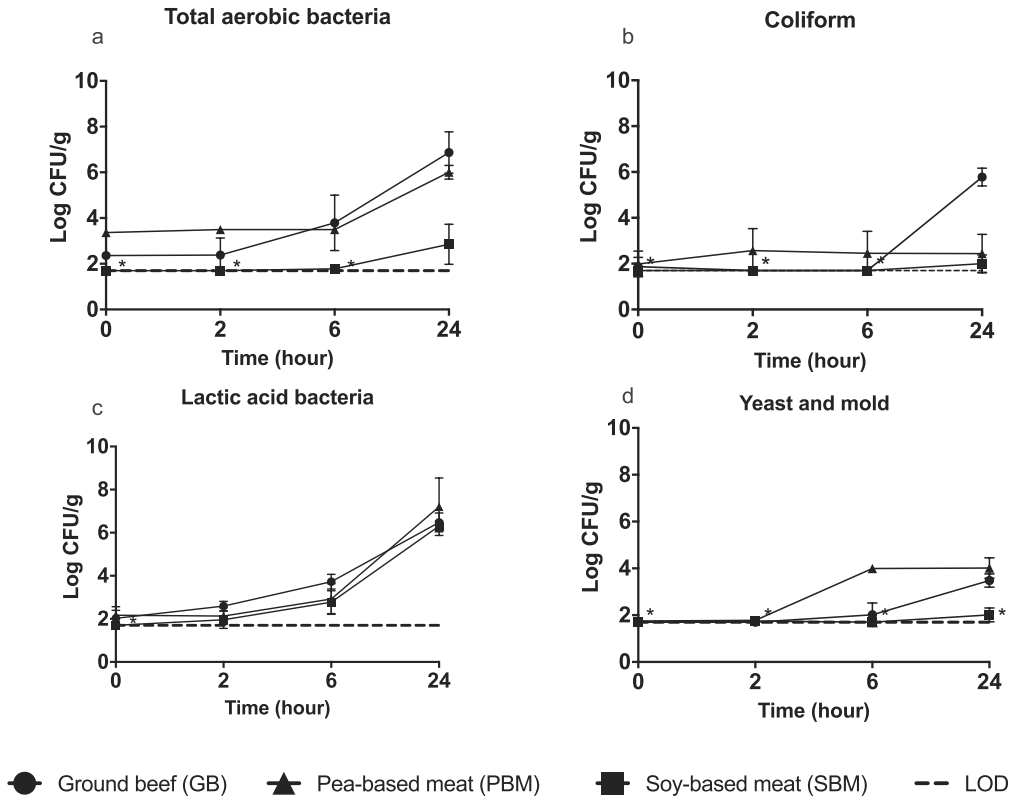

Fig. 2. Changes of the native microflora present in ground beef and plant-based meats during the 24-hour storage at 22 °C. (a) total aerobic bacteria, (b) coliform, (c) lactic acid bacteria, and (d) yeast and mold counts. *represents that the microbial counts of meat samples at that sampling point were below limit of enumeration (LOD = 2 Log CFU/g).

3.36 ± 0.17 log CFU/g respectively. Their initial coliform, LAB, and Y/M counts were also lower than the batches used for the 4 °C storage study. Differences seen in the initial background microflora levels were more significant for the different batches of GB than for SBM and PBM. For example, a 3-log difference in APC was observed between the GB used for the 4 °C storage study and the GB used for the 22 °C and 32 °C storage studies.

In general, the 24-hour temperature-abuse storage condition led to the increase of background microflora. By the end of storage at the ambient temperature (22 ℃), the average APC was 6.86 ± 0.90 and 6.00 ± 0.30 log CFU/g for GB and PBM. The APC in SBM was below the limit of enumeration (2.00 log CFU/g) for the first 6 h and increased to 2.85 ± 0.90 log CFU/g at the end of storage (Fig. 2**a**). A similar trend was observed for the coliform and Y/M levels in SBM; the levels of coliform and Y/M were below the limit of detection until Hour 6 and became countable, slightly greater than 2.00 log CFU/g at the end of storage (Fig. 2**d**). Although the coliform levels were below the limit of enumeration in GB for the first 6 h, 5.78 ± 0.38 log CFU/g of coliform were detected in GB on Hour 24 (Fig. 2**b**). The levels of LAB in all three types of meats gradually increased during storage; approximately 6.66 log CFU/g of LAB were observed in all meat types (Fig. 2**c**).

Greater increases of native microbial populations were observed in meat and meat analogues when the tested abuse temperature was 32 °C. Approximately 6.04, 4.32, and 3.80 log APC growth was observed in GB, PBM, and SBM respectively at the end of the storage period (Fig. 3**a**). For coliforms, 5.86 ± 0.16, 4.49 ± 1.40 and 3.24 ± 0.13 log CFU/g of coliform were observed in SBM, PBM, and GB respectively (Fig. 3**b**). The growth patterns of LAB in GB, SBM, and PBM were very similar, with 7.74 ± 0.20, 8.14 ± 0.40, and 8.28 ± 0.42 log CFU/g identified from them respectively (Fig. 3**c**). Higher numbers of Y/M counts were observed in GB and PBM than in SBM at the end of the 32 °C storage period (Fig. 3**d**).

3.3. Behavior of artificially-inoculated spoilage microorganisms in meat and meat analogues

The behavior of rifampicin-resistant *B. thermosphacta* and *P. fluorescens* artificially inoculated in meat and meat analogues when being held at 4 ℃ is shown in Fig. 4. The initial inoculation levels of *B. thermosphacta* were 3.70 ± 0.33, 3.46 ± 0.40, and 3.45 ± 0.17 log CFU/g for GB, SBM, and PBM, respectively (Fig. 4**a**). During the first five days of storage there was no significant change of *B. thermosphacta* levels regardless of the meat types (*P greater than 0*.05). From Day 5 to Day 10, the levels of *B. thermosphacta* significantly increased, with the final counts of 4.46 ± 0.35, 4.43 ± 0.28, and 5.03 ± 0.29 log CFU/g respectively for GB, SBM, and PBM. The initial inoculation levels of *P. fluorescens* were 2.98 ± 0.21, 3.15 ± 0.07, and 3.21 ± 0.07 log CFU/g for GB, SBM, and PBM. The growth of *P. fluorescens* was rapid in GB and PBM while the levels of *P. fluorescens* in SBM were not changed. Five days after storage, *P. fluorescens* in GB and PBM reached approximately 6.25 log CFU/g. The levels of *P. fluorescens* in GB continued increasing and reached 7.90 ± 0.27 log CFU/g by the end of storage. For both uninoculated and inoculated meat, the quality (color and smell) of the meat was deemed unacceptable at around Day 5 and Day 7 (**Figure S1**), regardless of the meat type. Thus, for the following pathogen inoculation study, 7 days was the time used for 4 °C storage studies.

3.4. Behavior of common foodborne pathogens in meat and meat analogues during storage

The behavior of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* artificially inoculated in meat and meat analogues when being held at 4 ℃ is reported in Table 2. The initial inoculation levels of *E. coli* O157:H7 were 2.70 ± 0.56, 3.07 ± 0.12, and 3.05 ± 0.19 log CFU/g in GB, SBM, and PBM respectively, based on the counts obtained from TSAR

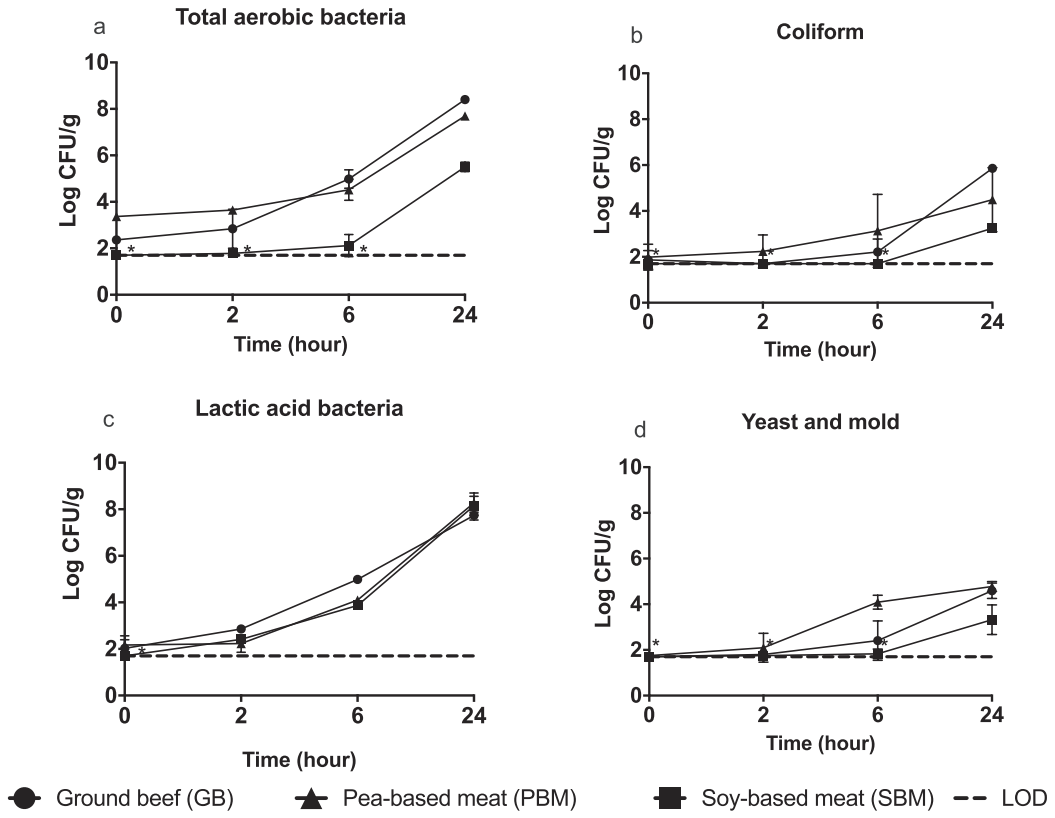

Fig. 3. Changes of the native microflora present in ground beef and plant-based meats during the 24-hour storage at 32 °C. (a) total aerobic bacteria, (b) coliform, (c) lactic acid bacteria, and (d) yeast and mold counts. *represents that the microbial counts of meat samples at that sampling point were below limit of enumeration (LOD = 2 Log CFU/g).

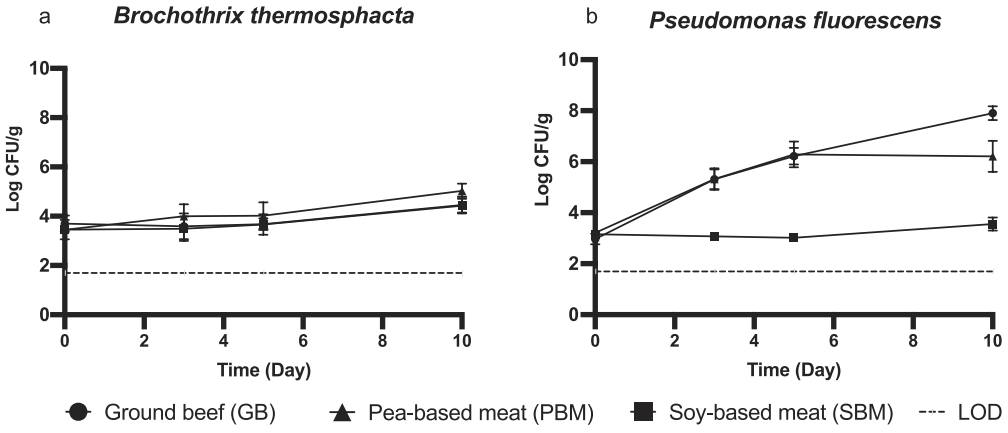

Fig. 4. Behavior of artificially-inoculated (a) *Brochothrix thermosphacta* and (b) *Pseudomonas fluorescens* in ground beef and plant-based meats during the 10-day storage at 4 °C.

(Table 2). Over the course of 7 days of storage, no significant (P greater than 0.05) change was observed in *E. coli* O157:H7 in GB and PBM. When looking at SBM, the levels of *E. coli* O157:H7 decreased ($P < 0.05$) on Day 7. For *Salmonella* and *L. monocytogenes*, no significant change of their population levels was observed during the 7-day storage in GB. In the plant-based meat analogues, the levels of *Salmonella* didn't change in either SBM or PBM during storage. The levels of *L. monocytogenes* increased in PBM (from 2.91 ± 0.14 log CFU/g to 3.65 ± 0.28 log CFU/g) during storage while remaining at the same level in SBM.

Pathogens grew in all meat types under abused temperatures (Tables 3 and 4). Faster growth was observed at 32 °C than at the ambient temperature. For *E. coli* O157:H7, the most rapid growth was observed in ground beef after 24 h. When inoculated in SBM and PBM, the growth of *E. coli* O157:H7 was more rapid in PBM than in SBM when stored at the ambient temperature. When the temperature was 32 °C, the final population of *E. coli* O157:H7 in SBM and PBM were 5.72 ± 0.10 and 5.43 ± 0.08 based on the counts obtained from TSAR. For *Salmonella* spp., the final populations in GB and PBM were similar regardless of the storage temperatures. For *L. monocytogenes*, the highest count after 24 h of storage was observed in PBM at 32 °C, and the lowest count was observed in SBM at 22 °C.

To better summarize the relationship between meat types, pathogen

Table 2
Behavior of pathogens inoculated in meat and meat analogues during the 7-day refrigeration storage at 4 °C (log CFU/g).

	GB		SBM		PBM	
***Escherichia coli* O157:H7**						
Day	TSAR	MACR	TSAR	MACR	TSAR	MACR
0	2.70 ± 0.56^{Aa}	2.78 ± 0.30^{Aa}	3.07 ± 0.12^{Aa}	2.80 ± 0.26^{Aa}	3.05 ± 0.19^{Aa}	2.66 ± 0.27^{Aa}
1	2.95 ± 0.21^{Aa}	2.71 ± 0.22^{Aab}	2.93 ± 0.13^{Aab}	2.32 ± 0.36^{Ab}	2.86 ± 0.23^{Aa}	2.57 ± 0.24^{Aa}
3	2.72 ± 0.20^{Aa}	2.30 ± 0.19^{Ac}	2.89 ± 0.25^{Aab}	2.26 ± 0.24^{Ab}	2.85 ± 0.28^{Aa}	2.51 ± 0.26^{Aa}
7	2.78 ± 0.14^{Aa}	2.34 ± 0.25^{ABbc}	2.75 ± 0.16^{Ab}	2.06 ± 0.09^{Bb}	2.83 ± 0.20^{Aa}	2.55 ± 0.23^{Aa}
***Salmonella* spp.**						
Day	TSAR	XLT4	TSAR	XLT4	TSAR	XLT4
0	3.43 ± 0.14^{ABa}	3.10 ± 0.28^{Aa}	3.26 ± 0.14^{Ba}	3.00 ± 0.26^{Aa}	3.46 ± 0.09^{Aa}	3.23 ± 0.23^{Aa}
1	3.22 ± 0.24^{Aa}	2.96 ± 0.38^{Aa}	3.38 ± 0.11^{Aa}	3.06 ± 0.18^{Aa}	3.46 ± 0.11^{Aa}	3.14 ± 0.26^{Aab}
3	3.39 ± 0.16^{Aa}	3.23 ± 0.22^{Aa}	3.39 ± 0.18^{Aa}	3.08 ± 0.29^{Aa}	3.43 ± 0.22^{Aa}	3.04 ± 0.18^{Aab}
7	3.20 ± 0.14^{Ba}	3.03 ± 0.15^{Aa}	3.46 ± 0.10^{Aa}	3.08 ± 0.13^{Aa}	3.23 ± 0.15^{Ba}	2.86 ± 0.19^{Ab}
Listeria monocytogenes						
Day	TSAR	MOX	TSAR	MOX	TSAR	MOX
0	2.61 ± 0.45^{Aa}	2.66 ± 0.42^{Aa}	2.71 ± 0.17^{Aa}	2.76 ± 0.12^{Aa}	2.91 ± 0.14^{Ab}	2.84 ± 0.10^{Ab}
1	2.53 ± 0.42^{Aa}	2.60 ± 0.23^{Aa}	2.54 ± 0.45^{Aa}	2.64 ± 0.24^{Aab}	2.64 ± 0.41^{Ab}	2.76 ± 0.15^{Ab}
3	2.83 ± 0.25^{Aa}	2.93 ± 0.19^{Aa}	2.44 ± 0.18^{Ba}	2.43 ± 0.18^{Bb}	2.96 ± 0.11^{Ab}	2.91 ± 0.13^{Ab}
7	2.80 ± 0.15^{Ba}	2.66 ± 0.17^{BA}	2.60 ± 0.12^{Ba}	2.64 ± 0.22^{Bab}	3.65 ± 0.20^{Aa}	3.61 ± 0.28^{Aa}

*Values are means ± standard deviations, n = 6. Different uppercase letters represent significant differences of cell counts among three meat types obtained from the same agar at each sampling point. Different lowercase letters represent significant differences of cell counts obtained from the same type of agar at different sampling points for the same type of meat or meat analogs ($P < 0.05$).

Table 3
Behavior of pathogens inoculated in meat and meat analogues during a 24-hour storage at 22 °C (log CFU/g).

	GB		SBM		PBM	
***Escherichia coli* O157:H7**						
Hour	TSAR	MACR	TSAR	MACR	TSAR	MACR
0	3.04 ± 0.15^{Aab}	3.03 ± 0.16^{Aa}	2.93 ± 0.13^{Aa}	2.79 ± 0.22^{Aa}	2.90 ± 0.05^{Aa}	2.65 ± 0.28^{Aa}
2	3.07 ± 0.13^{Ab}	3.00 ± 0.10^{Aa}	3.00 ± 0.09^{ABab}	2.57 ± 0.23^{Ba}	2.84 ± 0.15^{Ba}	2.59 ± 0.26^{Ba}
6	2.84 ± 0.12^{ABa}	2.47 ± 0.21^{Ab}	2.94 ± 0.04^{ABa}	2.55 ± 0.21^{Aa}	2.70 ± 0.22^{Ba}	2.40 ± 0.14^{Aa}
24	5.28 ± 0.10^{Ac}	5.08 ± 0.09^{Ac}	3.26 ± 0.32^{Bb}	2.98 ± 0.46^{Ba}	4.28 ± 0.14^{Cb}	4.20 ± 0.14^{Cb}
***Salmonella* spp.**						
Hour	TSAR	XLT4	TSAR	XLT4	TSAR	XLT4
0	3.34 ± 0.24^{Aa*}	3.32 ± 0.20^{Aa}	3.41 ± 0.15^{Aa}	3.26 ± 0.24^{Aa}	3.26 ± 0.30^{Aa}	3.24 ± 0.24^{Aa}
2	3.29 ± 0.38^{Aa}	3.24 ± 0.28^{Aa}	3.39 ± 0.24^{Aa}	3.31 ± 0.16^{Aa}	3.30 ± 0.21^{Aa}	3.28 ± 0.18^{Aa}
6	3.24 ± 0.16^{Aa}	3.18 ± 0.22^{Aa}	3.39 ± 0.24^{Aa}	3.28 ± 0.24^{Aa}	3.28 ± 0.26^{Aa}	3.34 ± 0.12^{Aa}
24	6.29 ± 0.17^{Ab}	5.56 ± 0.23^{Ab}	4.45 ± 0.30^{Bb}	4.23 ± 0.22^{Bb}	6.33 ± 0.32^{Ab}	6.18 ± 0.12^{Cb}
Listeria monocytogenes						
Hour	TSAR	MOX	TSAR	MOX	TSAR	MOX
0	2.92 ± 0.09^{Aa}	2.90 ± 0.20^{Aa}	2.74 ± 0.14^{Ba}	2.88 ± 0.08^{Aa}	2.91 ± 0.08^{Aba}	2.85 ± 0.30^{Aa}
2	2.99 ± 0.26^{Aa}	2.94 ± 0.25^{ABa}	2.90 ± 0.18^{Aa}	2.66 ± 0.30^{Aa}	3.00 ± 0.10^{Aa}	3.04 ± 0.08^{Ba}
6	3.08 ± 0.11^{Aba}	3.04 ± 0.21^{Ba}	2.85 ± 0.21^{Aa}	2.70 ± 0.26^{Aa}	3.24 ± 0.14^{Ba}	3.16 ± 0.16^{Ba}
24	5.12 ± 0.29^{Ab}	4.89 ± 0.11^{Bb}	3.86 ± 0.19^{Bb}	3.85 ± 0.18^{Ab}	4.56 ± 0.36^{Cb}	4.56 ± 0.40^{Bb}

*Values are means ± standard deviations, n = 6. Different uppercase letters represent significant differences of cell counts among three meat types obtained from the same agar at each sampling point. Different lowercase letters represent significant differences of cell counts obtained from the same type of agar at different sampling points for the same type of meat or meat analogs ($P < 0.05$).

types, and storage temperatures, primary and secondary models were established based on data presented in Tables 2, 3, and 4. Since there were four sampling points for each storage temperature, the linear regression model was chosen to better fit the curves. Parameters of the primary models are shown in **Table S1**. As indicated in **Table S1**, the linear model well fitted the data collected at 22 °C and 32 °C by showing higher R^2 (all greater than 0.90). However, the R^2 for GB at 4 °C were all low regardless of the pathogen types. The derivative secondary linear models were then built based on the primary model. Fig. 5 and Table 5 present the secondary model curves and parameter values associated with the secondary models. The growth kinetic constant (k) for *E. coli* O157:H7 in GB is 0.0083 log CFU/g per hour, which is significantly higher than k values for SBM and PBM. For *Salmonella* spp. the highest k value is also associated with GB. However, the k value of *Salmonella* in PBM is very close to GB. The lowest k value for *Salmonella* is found in SBM. For *L. monocytogenes*, the k values associated with GB and PBM are 0.0046 and 0.0047 log CFU/g per hour, respectively, indicating that the behavior of *L. monocytogenes* in GB and PBM is very similar. The lowest k value of *L. monocytogenes* is found in SBM. R^2 values for the secondary models are generally above 0.8, indicating the good fit of the linear model, except for the R^2 values describing *E. coli* O157:H7 behavior in SBM and *Salmonella* behavior in GB and PBM. Both the figure and the table indicate that pathogens grow the best in GB, followed by PBM and then SBM.

4. Discussion

The market for alternative meat or proteins was valued at $5.41 billion in 2021 and is expected to reach $12.3 billion by 2029 (Fortune business insights, 2021). Plant-based proteins, such as soy and pea proteins, have been the most widely used ingredients for the manufacturing of many major alternative meats on the market. Wild et al. (2014) pointed out that, given their neutral pH and high protein and moisture content, plant-based meats were as susceptible to microbial growth as traditional ground beef. To support the advancement of the plant-based meat industry, this study systematically evaluated the microbial quality and safety of plant-based meats by investigating the changes in the native microorganisms present in plant-based meats and monitoring the behavior of artificially inoculate spoilage and pathogenic bacteria during storage.

Our results showed that plant-based meat analogues, regardless of the type of plant protein, contained lower indigenous microbial loads compared to GB. The initial APC in GB was approximately 1 log higher than the other tested background microorganisms (i.e., yeast and mold, lactic acid bacteria, and coliforms). This result is in line with other reported data on microbial quality of ground beef or pork (Djordjević et al., 2019). The initial APC for plant-based meat was around 2–3 log CFU/g, and the variations between batch to batch were smaller compared to GB. The differences between GB and plant-based meat analogues were expected given the different production systems they have. More complex manufacturing methods and the involvement of heat treatment during the preparation of plant-based meats might be the reason for the lower initial APC counts. Extrusion, heating, cooling, drying and coagulation are processing technologies used to give plant-based meat its structure (Kyriakopoulou et al., 2019; Zhang et al., 2022). However, plant-based meat is not sterile; microorganisms can still be introduced to meat analogues through the addition of other raw ingredients or through post-processing contamination (Sampson et al., 2023). According to Lupo (2019) ingredients such as vitamins and

Table 4
Behavior of pathogens inoculated in meat and meat analogues during the 24-hour storage at 32 °C (log CFU/g).

	GB		SBM		PBM	
***Escherichia coli* O157:H7**						
Hour	TSAR	MACR	TSAR	MACR	TSAR	MACR
0	3.04 ± 0.15^{Aa}	2.93 ± 0.16^{Aa}	2.93 ± 0.13^{Aa}	2.79 ± 0.22^{Aa}	2.9 ± 0.52^{Aa}	2.65 ± 0.28^{Aa}
2	3.08 ± 0.18^{Aa}	2.72 ± 0.22^{Aa}	2.67 ± 0.32^{Ba}	2.40 ± 0.19^{Ba}	2.7 ± 0.18^{Ba}	2.29 ± 0.21^{Ba}
6	3.14 ± 0.38^{Aa}	2.57 ± 0.37^{Aa}	2.82 ± 0.17^{Aa}	2.6 ± 0.20^{Aa}	2.74 ± 0.41^{Aa}	2.48 ± 0.26^{Aa}
24	8.56 ± 0.04^{Ab}	8.02 ± 0.08^{Ab}	5.72 ± 0.10^{Bb}	5.26 ± 0.38^{Bb}	5.43 ± 0.08^{Cb}	4.95 ± 0.12^{Bb}
***Salmonella* spp.**						
Hour	TSAR	XLT4	TSAR	XLT4	TSAR	XLT4
0	3.34 ± 0.24^{Aa}	3.32 ± 0.20^{Aa}	3.40 ± 0.15^{Aa}	3.26 ± 0.24^{Aa}	3.26 ± 0.3^{Aa}	3.24 ± 0.24^{Aa}
2	3.38 ± 0.46^{Aa}	3.15 ± 0.17^{Aa}	3.40 ± 0.20^{Aa}	3.28 ± 0.27^{Aa}	3.28 ± 0.19^{Aa}	3.28 ± 0.20^{Aa}
6	3.74 ± 0.81^{Aa}	3.63 ± 0.11^{Aa}	3.26 ± 0.42^{Aa}	3.26 ± 0.35^{Aa}	3.63 ± 0.16^{Aa}	3.48 ± 0.24^{Aa}
24	7.82 ± 0.50^{Ab}	7.16 ± 0.64^{Ab}	7.36 ± 0.10^{Ab}	6.78 ± 0.56^{Ab}	7.88 ± 0.46^{Ab}	7.56 ± 0.34^{Ab}
Listeria monocytogenes						
Hour	TSAR	MOX	TSAR	MOX	TSAR	MOX
0	2.92 ± 0.09^{Aa}	2.90 ± 0.20^{Aa}	2.74 ± 0.15^{Ba}	2.88 ± 0.08^{Aa}	2.91 ± 0.08^{Aba}	2.85 ± 0.30^{Aa}
2	3.01 ± 0.12^{Aab}	2.89 ± 0.17^{Aa}	2.89 ± 0.10^{Aa}	2.95 ± 0.18^{ABa}	3.18 ± 0.07^{Bb}	3.17 ± 0.08^{Ba}
6	3.36 ± 0.25^{Ab}	3.26 ± 0.26^{Aa}	3.09 ± 0.23^{Aa}	2.93 ± 0.16^{Ba}	3.90 ± 0.06^{Bc}	3.86 ± 0.06^{Cb}
24	5.69 ± 0.36^{Ac}	5.54 ± 0.40^{Bb}	5.08 ± 0.54^{Bb}	4.93 ± 0.52^{Bb}	6.30 ± 0.20^{Cd}	6.16 ± 0.26^{Cc}

*Values are means ± standard deviations, n = 6. Different uppercase letters represent significant differences of cell counts among three meat types obtained from the same agar at each sampling point. Different lowercase letters represent significant differences of cell counts obtained from the same type of agar at different sampling points for the same type of meat or meat analogs ($P < 0.05$).

minerals, as well as flavoring and color, are added to plant-based meat to give it the desired sensory attributes. These ingredients do not go through thermal processing and may introduce microorganisms to the final products (Sampson et al., 2023).

Growth of background microorganisms was observed in all meat types during storage at three tested storage temperatures (4, 22, and 32 °C). Despite initial differences, the total LAB counts for all three meat types at the end of the storage were approaching or exceeding 7 log CFU/g, indicating that all meat types provided the nutrients needed for the growth of LAB. The growth of LAB led to the decreasing of pH in GB, regardless of the storage temperatures. The pH of PBM significantly decreased after 10 days of storage at 4 °C. When the storage temperature was at 22 °C, neither SBM nor PBM showed significant pH changes. When the storage temperature was set at 32 °C, pH of both SBM and PBM significantly decreased after 24 h. Taken together, the growth of LAB might not be the only factor contributing to the pH change in plant-based analogues. The difference in pH changes between GB and plant-based analogues maybe also attributed to the difference in their buffer capacity. The buffering capacity of food is determined by protein content, dipeptides, and the amount of acid/base groups of food matrix (Mennah-Govela et al., 2019; Puolanne and Kivikari, 2000; Salaün et al., 2005, Ebert et al., 2021). Mennah-Govela et al. (2019) showed that the buffering capacity of soy and pea protein was similar, as the total acid added to the 11 % soy dispersion and the 11 % pea dispersion was 194.9 ± 4.6 and 204.3 ± 2.1 μmol H^+ respectively for each gram of sample. In addition to pH changes, the appearance of the meat samples also changed as the storage time increased. For storage at the refrigerated condition, the color and smell of all meats was deemed unacceptable starting on Day 5 (Figure S1).

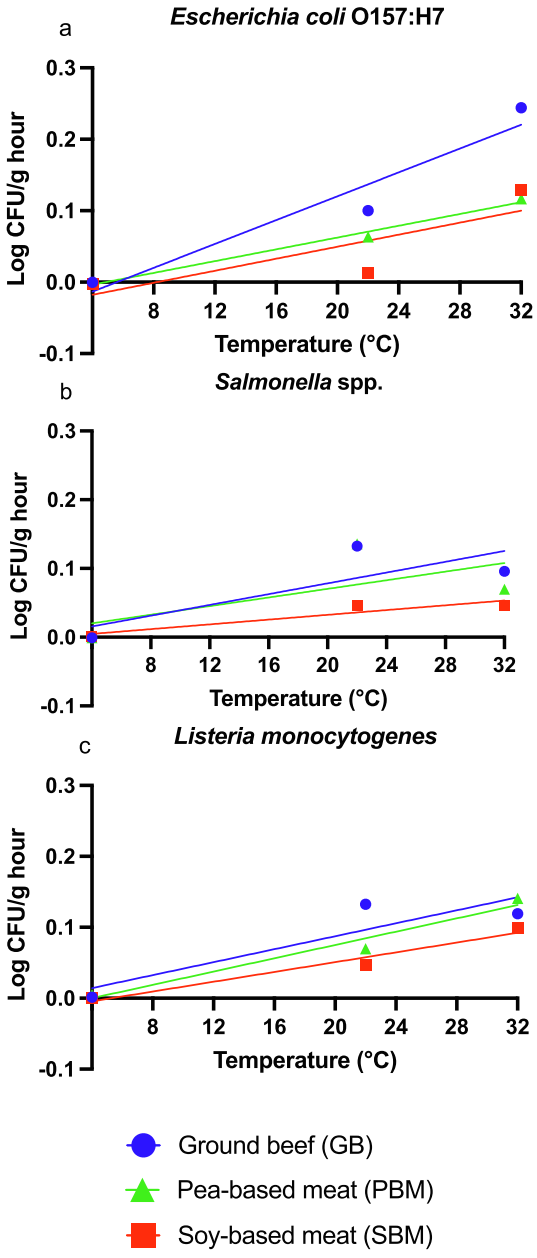

Fig. 5. Secondary models describing the behavior of pathogens artificially-inoculated in meat and meat analogues (a) *Escherichia coli,* (b) *Salmonella* spp., (c) *Listeria monocytogenes.*

Table 5
Parameters of the secondary models describing the survival and/or growth of pathogens in meat and meat analogues.

Parameters	R^2	*k*	*c*	RMSE
***Escherichia coli* O157:H7**				
GB	0.9308	0.0083	−0.0466	0.0458
SBM	0.6986	0.0042	−0.0344	0.0346
PBM	0.9881	0.0041	−0.0200	0.02
***Salmonella* spp.**				
GB	0.6525	0.0039	0.00006	0.0938
SBM	0.8733	0.0017	−0.0002	0.0283
PBM	0.4240	0.0031	0.0077	0.0173
Listeria monocytogenes				
GB	0.8089	0.0046	−0.004	0.0938
SBM	0.9606	0.0034	−0.0182	0.0282
PBM	0.9647	0.0047	−0.0186	0.0173

B. thermosphacta and *P. fluorescens* are dominant spoilage microorganisms for conventional meat products when stored under refrigerated conditions (Bahlinger et al., 2021; Doulgeraki et al., 2012; Pennacchia et al., 2011); Results of this study showed no significant difference was observed between the three types of meat regarding the levels of *B. thermosphacta* at the end of storage, GB was found to be the most supportive of *P. fluorescens* growth, followed by PBM. The levels of *P. fluorescens* in SBM did not increase significantly during the 10-day storage. The smallest increase of *P. fluorescens* seen in SBM compared to PBM or GB might due to the presence of soy protein. Fukao et al. (1998) reported that ca. 1.0 log CFU/g reduction of *P. fluorescens* was observed in kamaboko supplemented with 1 % soybean protein during the first 7 days of storage at 10 ℃, after that, an increase of ca. 3 log CFU/g was observed from Day 7 to Day 25. Further investigation about how *P. fluorescens* responds in SBM or soy protein-oriented food products need to be conducted. The supportive effect of GB on the growth of spoilage microorganisms has been well documented. Chung et al. (2000) showed that the levels of *P. fluoroscens* in beef steak stored at 5 ℃ for eight days increased by approximately 4 logs. Russo et al. (2006) showed that the level of *B. thermosphacta* increased by approximately 1.7–2.6 log CFU/g across all raw beef samples after seven days of storage at 5 ℃.

Salmonella enterica serovar Pratyphi B has been involved in an outbreak associated with unpasteurized tempeh, a fermented soybean product. This outbreak caused 89 cases in five states (Griese et al., 2013). To date, there have not been any recalls or outbreaks that directly connect shiga-toxin-producing *Escherichia coli* (STEC) or *L. monocytogenes* with plant-based proteins; one reason for that might be because only a small proportion of the population consumes plant-based products (Porto-Fett et al., 2020; Slade, 2018). However, as discussed earlier, microorganisms can be introduced to the final products through raw ingredients or packaging and handling steps after manufacturing. We found that both SBM and PBM supported the growth of foodborne pathogens. This supportive effect is depending on the temperature, types of pathogens and meat. For example, when being stored at 22 or 32 °C for 24 h, *E. coli* O157:H7 grew better in GB than in SBM and PBM. For *Salmonella* and *L. monocytogenes*, PBM supported the growth of these two pathogens better than SBM. When being stored at 4 °C, PBM supported the growth of *L. monocytogenes* better (ca. 0.74-log growth was observed after 7 days of storage) compared to GB. Similar observations were made by Luchansky et al. (2020). In this study, the authors showed that *L. monocytogenes* increased 1.3 log CFU/g in plant-based meat after 21-day storage at 4 °C, while the levels of *L. monocytogenes* did not change in GB. Although study performed by Luchansky et al. (2020) did not indicate whether the plant-based meat was pea-based or soy-based, both the present study and Luchansky et al. (2020) proved that plant-based meat could support the growth of common foodborne pathogens, including *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*. The better growth of *L. monocytogenes* in PBM compared with GB may be due to the presence of lower levels of background microorganisms during 4 °C storage. The difference in the behavior of *L. monocytogenes* in PBM and SBM suggested that ingredients of these two types of plant-based meat may also lead to different behaviors of *L. monocytogenes*. The linear regression model was used in this study for the establishment of predictive models due to the poor fit of other models. The application of the linear model in predictive microbiology has been shown to be reliable, especially when experimental data is limited (Buchanan et al., 1997). Linear microbial predictive models have been used previously to predict the growth of *Listeria monocytogenes* in ready-to-eat foods and the survival of common foodborne pathogens in dried fruits (Canakapalli et al., 2022; FDA, 2003; Pouillot and Lubran, 2011; Skjerdal et al., 2021). Based on the prediction, pathogens grow the best in GB, followed by PBM and then SBM. To provide a more comprehensive prediction on complex food such as plant-based meat analogues, factors including dynamic temperature/pH change, microbial interactions, and types of plant-based protein should be considered for the future model development.

5. Conclusion

The market for plant-based meats is expected to continue expanding in the next decade. Therefore, building a solid foundation of knowledge about the microbial quality and safety of plant-based meat analogues is needed to support this industry's sustainable growth and advancement. This study showed that, although some thermal processing steps are included in the preparation of plant-based meat, microorganisms can still be introduced into plant-based meat through untreated raw ingredients or through environmental contamination. Once introduced into plant-based meat analogues, both spoilage and pathogenic microorganisms can survive and even grow in meat analogues. Storage temperatures, type of pathogen and spoilage microorganisms, and levels of native microorganisms, as well as the plant protein types, can all impact the behavior of different microorganisms. Control strategies, ranging from control and certification of suppliers to environmental monitoring, are needed in order to ensure the safety and quality of plant-based meat analogues.

CRediT authorship contribution statement

Zhuosheng Liu: Methodology, Data curation, Formal analysis, Writing - original draft. **Maria Shaposhnikov:** Methodology, Data curation, Formal analysis, Writing - original draft. **Shuai Zhuang:** Writing - original draft. **Tianyi Tu:** Data curation. **Hongye Wang:** Conceptualization, supervision, writing – review and editing. **Luxin Wang:** Conceptualization, supervision, writing – review and editing.

Declaration of Competing 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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.112408.

References

Andrews, W. H., Jacobson, A., & Hammack, T. (2018). Bacteriological analytical manual (BAM) chapter 5: *Salmonella*. *Bacteriological Analytical Manual, 110*.

Bahlinger, E., Dorn-In, S., Beindorf, P.-M., Mang, S., Kaltner, F., Gottschalk, C., … Schwaiger, K. (2021). Development of two specific multiplex qPCRs to determine amounts of *Pseudomonas*, *Enterobacteriaceae*, *Brochothrix thermosphacta* and *Staphylococcus* in meat and heat-treated meat products. *International Journal of Food Microbiology, 337*, Article 108932.

Bakhsh, A., Lee, S.-J., Lee, E.-Y., Sabikun, N., Hwang, Y.-H., & Joo, S.-T. (2021). A novel approach for tuning the physicochemical, textural, and sensory characteristics of plant-based meat analogs with different levels of methylcellulose concentration. *Foods, 10*(3), 560.

Boukid, F., Rosell, C. M., & Castellari, M. (2021). Pea protein ingredients: A mainstream ingredient to (re) formulate innovative foods and beverages. *Trends in Food Science & Technology, 110*, 729–742.

Buchanan, R., Whiting, R., & Damert, W. (1997). When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiology, 14*(4), 313–326.

Canakapalli, S. S., Sheng, L., & Wang, L. (2022). Survival of common foodborne pathogens on dates, sundried tomatoes, and dried pluots at refrigerated and ambient temperatures. *LWT, 154*, Article 112632.

Casaburi, A., Piombino, P., Nychas, G.-J., Villani, F., & Ercolini, D. (2015). Bacterial populations and the volatilome associated to meat spoilage. *Food Microbiology, 45*, 83–102.

Chaitiemwong, N., Hazeleger, W., Beumer, R., & Zwietering, M. (2014). Quantification of transfer of *Listeria monocytogenes* between cooked ham and slicing machine surfaces. *Food Control, 44*, 177–184.

Chung, M.-S., Ko, Y.-T., & Kim, W.-S. (2000). Survival of *Pseudomonas fluorescens* and *Salmonella Typhimurium* after electron beam and gamma irradiation of refrigerated beef. *Journal of Food Protection, 63*(2), 162–166.

Demetrakakes, P. (2022). Report: Beyond meat plant had sanitation problems. Available at https://www.foodprocessing.com/food-safety/cleaning-sanitation-hygiene/news/21438201/report-beyond-meat-plant-had-sanitation-problems Accessed on December 10, 2022.

Djordjević, J., Bošković, M., Lazić, I. B., Djordjević, V., Baltić, T., Laudanović, M., & Baltić, M.Ž. (2019). Spoilage-related bacteria of pork and beef minced meat under vacuum and modified atmosphere. *Romanian Biotechnological Letters, 24*(4), 658–668.

Doulgeraki, A. I., Ercolini, D., Villani, F., & Nychas, G.-J.-E. (2012). Spoilage microbiota associated to the storage of raw meat in different conditions. *International Journal of Food Microbiology, 157*(2), 130–141.

Ebert, S., Baune, M.-C., Broucke, K., Van Royen, G., Terjung, N., Gibis, M., & Weiss, J. (2021). Buffering capacity of wet texturized plant proteins in comparison to pork meat. *Food Research International, 150*, Article 110803.

FDA. (2003). Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods Available at https://www.fda.gov/food/cfsan-risk-safety-assessments/quantitative-assessment-relative-risk-public-health-foodborne-listeria-monocytogenes-among-selected Accessed on October, 20th, 2022.

Feng, P., Weagant, S., & Karen, J. (2020). BAM Chapter 4A: Diarrheagenic *Escherichia Coli*. *Bacteriological Analytical Manual*.

Fortune Business Insights. (2021). Market Research Report. Available at: https://www.fortunebusinessinsights.com/industry-reports/meat-substitutes-market-100239 Accessed on October 6th 2022.

Fukao, T., Ohyabu, S., Sawada, H., & Ohta, Y. (1998). Effects of addition of powdered protein to Kamaboko on growth of *Pseudomonas fluorescens*. *Fisheries Science, 64*(1), 57–61.

Griese, S. E., Fleischauer, A. T., MacFarquhar, J. K., Moore, Z., Harrelson, C., Valiani, A., … Griffin, D. (2013). Gastroenteritis outbreak associated with unpasteurized tempeh, North Carolina, USA. *Emerging Infectious Diseases, 19*(9), 1514.

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, 10*(6), 1226.

Hitchins, A. D., Jinneman, K., & Chen, Y. (2017). BAM Chapter 10: Detection of *Listeria monocytogenes* in foods and environmental samples, and enumeration of *Listeria monocytogenes* in foods. *Bacteriological Analytical Manual* 99-103.

Hu, P., Zhou, G., Xu, X., Li, C., & Han, Y. (2009). Characterization of the predominant spoilage bacteria in sliced vacuum-packed cooked ham based on 16S rDNA-DGGE. *Food Control, 20*(2), 99–104.

Iulietto, M. F., Sechi, P., Borgogni, E., & Cenci-Goga, B. T. (2015). Meat spoilage: A critical review of a neglected alteration due to ropy slime producing bacteria. *Italian Journal of Animal Science, 14*(3), 4011.

Kröckel, L. (2013). The role of lactic acid bacteria in safety and flavour development of meat and meat products. *Lactic Acid Bacteria-R & D for Food, Health and Livestock Purposes*.

Kyriakopoulou, K., Dekkers, B., & van der Goot, A. J. (2019). Plant-based meat analogues. In *Sustainable Mmeat Production and Processing* (pp. 103–126). Elsevier.

Liu, Z., Liao, C., Golson, K., Phillips, S., & Wang, L. (2021). Survival of common foodborne pathogens on dried apricots made with and without sulfur dioxide treatment. *Food Control, 121*, Article 107569.

Liu, Z., Sheng, L., Canakapalli, S. S., & Wang, L. (2022). Evaluation of the microbial control efficacies of commonly used home-drying and storage practices of dried peaches. *LWT, 165*, Article 113734.

Luchansky, J. B., Shoyer, B. A., Jung, Y., Shane, L. E., Osoria, M., & Porto-Fett, A. C. (2020). Viability of Shiga toxin–producing *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* Within plant versus beef burgers during cold storage and following pan frying. *Journal of Food Protection, 83*(3), 434–442.

Lupo, L. (2019). The impossible burger. Available at: https://www.qualityassurancemag.com/article/the-impossible-burger/. Accessed on October 17, 2022.

Mennah-Govela, Y. A., Singh, R. P., & Bornhorst, G. M. (2019). Buffering capacity of protein-based model food systems in the context of gastric digestion. *Food and Function, 10*(9), 6074–6087.

Omer, M. K., Alvarez-Ordonez, A., Prieto, M., Skjerve, E., Asehun, T., & Alvseike, O. A. (2018). A systematic review of bacterial foodborne outbreaks related to red meat and meat products. *Foodborne Pathogens and Disease, 15*(10), 598–611.

Pennacchia, C., Ercolini, D., & Villani, F. (2011). Spoilage-related microbiota associated with chilled beef stored in air or vacuum pack. *Food Microbiology, 28*(1), 84–93.

Pohlman, F. W., Stivarius, M. R., McElyea, K. S., Johnson, Z. B., & Johnson, M. G. (2002). The effects of ozone, chlorine dioxide, cetylpyridinium chloride and trisodium phosphate as multiple antimicrobial interventions on microbiological, instrumental color, and sensory color and odor characteristics of ground beef. *Meat Science, 61*(3), 307–313.

Porto-Fett, A., Shane, L. E., Shoyer, B. A., Osoria, M., Jung, Y., & Luchansky, J. B. (2020). Inactivation of Shiga toxin–producing *Escherichia coli* and *Listeria monocytogenes* within plant versus beef burgers in response to high pressure processing. *Journal of Food Protection, 83*(5), 865–873.

Pouillot, R., & Lubran, M. B. (2011). Predictive microbiology models vs. modeling microbial growth within *Listeria monocytogenes* risk assessment: What parameters matter and why. *Food Microbiology, 28*(4), 720–726.

Puolanne, E., & Kivikari, R. (2000). Determination of the buffering capacity of postrigor meat. *Meat Science, 56*(1), 7–13.

Russo, F., Ercolini, D., Mauriello, G., & Villani, F. (2006). Behaviour of *Brochothrix thermosphacta* in presence of other meat spoilage microbial groups. *Food Microbiology, 23*(8), 797–802.

Salaün, F., Mietton, B., & Gaucheron, F. (2005). Buffering capacity of dairy products. *International Dairy Journal, 15*(2), 95–109.

Sampson, G. L., Ruelle, S. B., Phan, L., Williams-Hill, D., & Hellberg, R. S. (2023). Effectiveness of selected pre-enrichment broths for the detection of Salmonella spp. in meat analogs. *Food Control, 143*, 109282.

Skjerdal, T., Gangsei, L. E., Alvseike, O., Kausrud, K., De Cesare, A., Alexa, E.-A., … From, C. (2021). Development and validation of a regression model for *Listeria monocytogenes* growth in roast beefs. *Food Microbiology, 98*, Article 103770.

Slade, P. (2018). If you build it, will they eat it? Consumer preferences for plant-based and cultured meat burgers. *Appetite, 125*, 428–437.

Smith, J., Benedict, R., & Palumbo, S. (1982). Protection against heat-injury in *Staphylococcus aureus* by solutes. *Journal of Food Protection, 45*(1), 54–58.

Sofos, J. N., & Geornaras, I. (2010). Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157: H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. *Meat Science, 86*(1), 2–14.

Tóth, A. J., Dunay, A., Battay, M., Illés, C. B., Bittsánszky, A., & Süth, M. (2021). Microbial Spoilage of Plant-Based Meat Analogues. *Applied Sciences, 11*(18), 8309.

USDA. (1998). Physical examination of meat and poultry products. Available at https://fsis-prod.fsis.usda.gov/sites/default/files/media_file/2021-03/MLG-2.pdf Accessed on July, 3, 2022.

USDA. (2016). Ground Beef and Food Safety. Available at https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/meat/ground-beef-and-food-safety Accessed on July, 3, 2022.

Wild, F., Czerny, M., Janssen, A. M., Kole, A., Zunabovic, M., & Domig, K. J. (2014). The evolution of a plant-based alternative to meat. *Agro FOOD Industry Hi Tech, 25*(1), 45–49.

Xiang, N., Lyu, Y., Zhu, X., Bhunia, A. K., & Narsimhan, G. (2017). Effect of physicochemical properties of peptides from soy protein on their antimicrobial activity. *Peptides, 94*, 10–18.

Xu, J., Zhang, P., Zhuang, L., Zhang, D., Qi, K., Dou, X., … Gong, J. (2019). Multiplex polymerase chain reaction to detect *Salmonella* serovars Indiana, Enteritidis, and Typhimurium in raw meat. *Journal of Food Safety, 39*(5), e12674.

Zhang, C., Guan, X., Yu, S., Zhou, J., & Chen, J. (2022). Production of meat alternatives using live cells, cultures and plant proteins. *Current Opinion in Food Science, 43*, 43–52.