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Microbiological and antinutritional risks in plant-based alternatives on the Swedish market

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ABSTRACT

Rising consumer demand and environmental concerns have accelerated the market for plant-based meat and dairy alternatives (PBAs). However, limited research is available regarding their microbiological safety and nutritional quality. A total of 40 PBAs available on the Swedish market (39 vegan, 1 vegetarian) and one cow's milk reference were analyzed for microbial composition, phytic acid, iron content, and estimated iron bioavailability. Total aerobic counts, *Enterobacteriaceae*, yeasts and molds, lactic acid bacteria, and spore-forming *Bacillus* spp. were quantified, and isolates were identified using Sanger and next generation sequencing. Dairy analogues contained a higher proportion of spore-forming bacteria, whereas meat analogues exhibited greater *Bacillus* diversity. Lactic acid bacteria were common, particularly in pea-, bean-, and soy-based products. Sequencing also detected genera commonly associated with human oral and gut microbiota, including *Campylobacter*, *Prevotella*, and *Rothia*. Phytic acid and iron contents varied among products, with ingredient source strongly influencing their levels. Most samples exhibited relatively high phytic acid-to-iron molar ratios, indicating a low estimated iron bioavailability. These findings support improved quality-control strategies and nutritional evaluation of PBAs.

1. Introduction

In many high-income countries, animal-source foods contribute a substantial proportion of total dietary protein intake. However, growing environmental concerns and changing consumer preferences have increased interest in plant-based protein products (Aschemann-Witzel et al., 2021; Trindler et al., 2022). With the global population projected to reach 10 billion by 2050, sustainable protein production has become a central challenge for food systems (United Nations Population Fund, 2024). Animal-based foods, particularly red meat and dairy products, are associated with high greenhouse gas emissions compared to plant-based alternatives (PBAs).

Over the past decade, the market for plant-based foods has expanded rapidly, particularly plant-based meat and dairy alternatives. The global plant-based food market was valued at approximately USD 14 billion in 2025 and is expected to reach USD 44 billion by 2035, reflecting a compound annual growth rate of 12% (Future Market Insights Inc.,

2026). Initially, these products mainly targeted consumers reducing animal protein intake for ethical, environmental, or health reasons. Today, an increasing number of consumers incorporate plant-based products into their daily diets due to the perception that they are inherently healthier than animal-based foods (Hemler & Hu, 2019; Possidônio et al., 2021). PBAs are often marketed as direct replacements for meat and dairy, enabling a gradual transition toward a more sustainable diet. While plant foods like whole grains, vegetables, legumes and seeds have well-documented health benefits, concerns have emerged regarding the nutritional, microbiological, and chemical safety of highly processed PBAs. Some products contain elevated levels of salt, saturated fat, and refined carbohydrates, and undergo complex manufacturing processes that may introduce new food safety challenges (Boukid et al., 2021; McClements & Grossmann, 2021).

Despite their increasing popularity, PBAs have raised concerns related to microbiological safety, and overall food quality (Liu et al., 2023; Tóth et al., 2021). Many of these concerns arise from the

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novelty of plant-based protein ingredients and the limited understanding of their associated microbiological risks (Hadi & Brightwell, 2021). Plant-based meat alternatives typically contain high levels of protein and moisture and often exhibit a near-neutral pH, creating favorable conditions for the growth of spoilage microorganisms and foodborne pathogens (Liu et al., 2023; Luchansky et al., 2020). Storage conditions such as temperature, humidity, and packaging atmosphere further influence microbial growth and product quality. In addition, spore-forming bacteria such as *Bacillus* spp. cause challenges in heat-treated foods due to their ability to survive thermal processing and produce enterotoxins at low infectious doses (Helstad et al., 2023; Voss et al., 2018). Documented foodborne outbreaks illustrate the microbiological risks associated with plant-based products. A multistate outbreak of *Salmonella stanley* associated with vegan cheese in the United States involved 17 cases, including 3 hospitalizations (CDC, 2024). In addition, a 2024 Canadian outbreak of *Listeria monocytogenes* associated with plant-based milk involved 18 cases, 13 hospitalizations, and 2 deaths (FSN, 2024).

In addition to microbiological considerations, the presence of anti-nutrients is another concern in plant-based products. Antinutrients are compounds that have vital roles in plants, primarily acting as chemical defense against herbivores, insects, pests, and predators. Despite their essential roles in plants, some of these compounds can also reduce nutrient absorption in humans. Common antinutrients include oxalates, lectins, tannins, saponins, and phytic acid (PA) (López-Moreno et al., 2022; Phan et al., 2018). PA, also known as myo-inositol-1,2,3,4,5,6-hexakisphosphate (IP6), is widely present in grains, legumes, and seeds, where it functions as the main storage form for phosphorus and inositol in plant seeds (Kumar et al., 2010; Loewus, 2001). Due to its strong chelating properties, PA can form insoluble complexes with minerals such as iron, zinc, and calcium, thereby reducing their bioavailability (Sarkhel & Roy, 2022; Thavarajah et al., 2010). Although *in vitro* and animal studies suggest that PA may exhibit biological activity, evidence for clinically relevant effects in humans remains limited (Lan et al., 2022; Silva & Bracarense, 2016). The nutritional impact of PA depends on dietary context, food processing, and the overall balance of mineral intake.

Iron is an essential nutrient for human health, playing a critical role in numerous physiological functions. It is involved in DNA synthesis and repair, mitochondrial processes, and the production of oxygen-transport proteins such as hemoglobin and myoglobin. Additionally, iron is required for the formation of enzymes, including heme enzymes, and for the production of new blood cells (Abbaspour et al., 2014; McDowell, 2003; Paul et al., 2017). Despite its fundamental biological importance, ensuring adequate iron intake and uptake remains a public health concern, particularly in the context of shifting dietary patterns.

Nutrient adequacy in plant-based diets has also become a public health concern. A recent Swedish study involving 475 teenage girls reported that 38.1% of participants were iron deficient, with substantially higher prevalence among vegetarians/vegans (69.4%) and pescatarians (49.4%) compared to omnivores (30.5%) (Stubbendorff et al., 2025). Similarly, Hallström et al. (2025) reported that among Swedish adolescents aged 11–18 years, diets with higher iron intake, particularly heme iron were associated with greater climate impact, whereas lower climate-impact diets were linked to an increased risk of iron deficiency in girls. Together, these findings underscore the importance of evaluating not only iron content but also iron bioavailability in plant-based products that increasingly replace animal-derived foods.

Despite rapid market growth, research on the microbiological safety and nutritional quality of PBAs remains limited. Therefore, this study investigated microbiological composition, phytic acid content, iron content, and estimated iron bioavailability in a broad range of plant-based products representing multiple categories on the Swedish market. Microbial communities were characterized using culture-dependent methods, Sanger sequencing, and next-generation sequencing, while iron bioavailability was estimated using the phytic acid-to-iron molar

ratio. Given the increasing role of PBAs in sustainable dietary transitions, better understanding of their microbiological and nutritional characteristics is essential.

2. Materials and methods

2.1. Study design

A total of 40 commercially available PBAs and one conventional cow's milk reference sample were purchased from major retail stores in Lund, Sweden. The collection included 39 vegan PBAs, 1 vegetarian product (MA6), representing 25 brands. The 40 PBAs comprised 34 meat alternatives and 6 dairy alternatives. According to processing type, the samples consisted of cold cuts/others ($n = 6$), fillets ($n = 8$), formulated non-heat-treated products ($n = 3$), formulated heat-treated products ($n = 8$), formulated heat-treated products with visible chunks ($n = 2$), homogeneous non-heat-treated mince products ($n = 2$), homogeneous pre-cooked mince products ($n = 1$), non-homogeneous heat-treated mince products ($n = 4$), and beverages ($n = 6$). According to storage conditions, 20 PBAs were refrigerated, 14 were frozen, and 6 were stored at ambient temperature. The main ingredient groups comprised peas ($n = 11$), soy ($n = 7$), beans ($n = 6$), mixed/other legumes ($n = 5$), wheat/gluten ($n = 5$), mycoprotein ($n = 2$), oats ($n = 2$), nuts ($n = 1$), and egg/dairy ($n = 1$). One conventional cow's milk sample was included separately as a reference.

Products were categorized according to storage condition, main ingredient source, and processing type. Processing categories were defined based on product label information and visual product characteristics. These categories included homogeneous mince (non-heat-treated or heat-treated), non-homogeneous mince (heat-treated), formulated products (non-heat-treated or heat-treated), formulated products with visible chunks, fillets, cold cuts, and beverages.

Homogeneous products were defined as products with a uniform internal structure in which individual ingredients were not visually distinguishable, whereas non-homogeneous products contained visible particles or fragments of raw materials. Formulated products referred to products shaped to resemble conventional meat products such as burgers, sausages, or nuggets, typically containing additional stabilizing ingredients. Fillet products consisted of intact extruded structures without added stabilizers, while cold cuts referred to ready-to-eat sliced products. Plant-based milk alternatives were classified as beverages.

The categorization strategy was developed to capture differences in product structure, processing intensity, and expected microbial exposure.

Following purchase, all products were stored unopened according to the storage conditions specified on the product labels (frozen, refrigerated, or ambient temperature; Table 1A and Table 1B) until analysis. Frozen products were thawed at room temperature for 1 h immediately prior to analysis. All products were analyzed at least one week prior to their stated best-before date to minimize potential effects of shelf-life variation on microbiological and nutritional measurements.

2.2. Sample preparation for microbiological analysis

For preparation, 10 g of each sample was mixed with 90 mL of sterile bacteriological peptone water (NaCl, Merck, 8.5 g/L; Bacteriological peptone, Oxoid, 1 g/L). The mixture was then homogenized for 5 min at maximum speed in a Stomacher® 400 (Seward, UK). The resulting suspension was then distributed into three Eppendorf tubes and one centrifuge tube. The contents of the Eppendorf tubes were filtered using 5 µm surfactant-free cellulose acetate syringe filters (Sartorius, Germany) and subsequently used for microbial enumeration. The centrifuge tube was stored at -20°C for downstream Sanger- and next-generation sequencing analysis.

An overview of the experimental design is shown in Supplementary Data, Fig. S1.

Table 1A

Investigated plant-based **Meat Alternatives (MA)** in the Swedish market. The table summarizes the processing type, product name, main ingredient source & storage condition for each product included in the study.

No	Sample code	Processing type	Product name	Main ingredient source	Storage condition
1	MA2	Cold cuts/others	Vegan ham	Peas	Refrigerated
2	MA4	Cold cuts/others	Vegan salami	Mixed/Other legumes	Refrigerated
3	MA6	Cold cuts/others	Vegetarian sausage A	Egg/Dairy	Refrigerated
4	MA7	Cold cuts/others	Vegan roast beef	Peas	Refrigerated
5	MA15	Cold cuts/others	Vegan smoked slices	Mycoprotein	Refrigerated
6	MA16	Cold cuts/others	Vegan pepperoni	Mycoprotein	Refrigerated
7	MA3	Fillet	Vegan kebab A	Wheat/Gluten	Refrigerated
8	MA9	Fillet	Vegan chicken breast	Mixed/Other legumes	Refrigerated
9	MA11	Fillet	Vegan kebab B	Wheat/Gluten	Refrigerated
10	MA12	Fillet	Vegan mini fillet	Soy	Refrigerated
11	MA13	Fillet	Vegan kebab C	Wheat/Gluten	Refrigerated
12	MA18	Fillet	Seitan block	Wheat/Gluten	Refrigerated
13	MA22	Fillet	Wheat flakes	Wheat/Gluten	Frozen
14	MA29	Fillet	Pulled beans	Soy	Frozen
15	MA10	Formulated not pre-heated	Vegan burger A	Peas	Refrigerated
16	MA21	Formulated not pre-heated	Vegan burger B	Peas	Frozen
17	MA25	Formulated not pre-heated	Vegan burger D	Peas	Frozen
18	MA5	Formulated pre-heated	Vegan nuggets	Mixed/Other legumes	Refrigerated
19	MA8	Formulated pre-heated	Vegan meatballs	Mixed/Other legumes	Refrigerated
20	MA14	Formulated pre-heated	Vegan sausage B	Mixed/Other legumes	Refrigerated
21	MA17	Formulated pre-heated	Vegan sausage C	Beans	Refrigerated
22	MA19	Formulated pre-heated	Vegan beef	Beans	Refrigerated
23	MA20	Formulated pre-heated	Vegan sausage D	Peas	Refrigerated
24	MA26	Formulated pre-heated	Vegan burger E	Peas	Frozen
25	MA27	Formulated pre-heated	Vegan burger F	Soy	Frozen
26	MA24	Formulated pre-heated chunks	Vegan burger C	Beans	Frozen
27	MA28	Formulated pre-heated chunks	Vegan burger G	Peas	Frozen
28	MA1	Mince homogeneous not pre-heated	Vegan mince A	Peas	Refrigerated
29	MA23	Mince homogeneous not pre-heated	Vegan mince B	Peas	Frozen
30	MA30	Mince homogeneous pre-cooked	Vegan mince C	Beans	Frozen
31	MA31	Mince not homogeneous pre heated	Vegan mince D	Beans	Frozen

Table 1A (continued)

No	Sample code	Processing type	Product name	Main ingredient source	Storage condition
32	MA32	Mince not homogeneous pre heated	Vegan mince E	Soy	Frozen
33	MA33	Mince not homogeneous pre heated	Vegan mince F	Soy	Frozen
34	MA41	Mince not homogeneous pre heated	Vegan mince G	Soy	Frozen

Table 1B

Investigated plant-based **Dairy Alternatives (DA)** in the Swedish market. The table summarizes the processing type, product name, main ingredient source & storage condition, for each product included in the study.

No	Sample code	Processing type	Product name	Main ingredient source	Storage condition
35	DA34	Beverages	Soy drink	Soy	Room temperature
36	DA35	Beverages	Almond drink	Nuts	Room temperature
37	DA36	Beverages	Fava bean drink	Beans	Room temperature
38	DA37	Beverages	Oat drink 1.5%	Cereals (oats)	Room temperature
39	DA38	Beverages	Oat drink 3%	Cereals (oats)	Room temperature
40	DA39	Beverages	Pea drink	Peas	Room temperature
41	SM40	Beverages	Standard milk 3%	Egg/Dairy	Refrigerated

^aOne conventional dairy product (standard cow's milk) was included in this group as a reference sample for comparative purposes.

2.3. Microbial enumeration and isolation

Viable counts were performed on the 41 homogenized food samples. A 100 µL aliquot of the homogenized sample was immediately spread onto duplicate agar plates using glass beads. The following selective and non-selective media were used: Tryptic Soy Agar (TSA), (Merck Millipore, Darmstadt, Germany) for total aerobic count, Violet Red Bile Dextrose Agar (VRBD), (Merck Millipore, Darmstadt, Germany) for Enterobacteriaceae count, Malt Extract Agar (MA), (Merck Millipore, Darmstadt, Germany) for yeast and mold, as well as De Man, Rogosa, Sharpe Agar (MRS), (Merck Millipore, Darmstadt, Germany), for lactic acid bacteria count, and Bacillus ChromoSelect Agar (BCA) (Sigma-Aldrich, MO, USA) for spore-forming *Bacillus* spp.

The incubation conditions for the different media were as follows: BCA, TSA, and MA plates were incubated aerobically at 30°C for 2, 3, and 5 days, respectively. Meanwhile, MRS and VRBD plates were incubated aerobically at 37°C for 3 days.

Spore activation was performed according to Krawczyk et al. (2017) with modifications. Homogenized samples were incubated in water baths at 60°C for 6- and 16-h prior to plating onto BCA (Sigma-Aldrich, MO, USA) for the enumeration of spore-forming *Bacillus* spp. These incubation conditions were selected to investigate the survival and activation of spore-forming bacteria under prolonged heat exposure relevant to food warm-holding and potential temperature-abuse scenarios.

The limit of detection (LOD) for microbial enumeration was 2 log₁₀ CFU/g. The upper limit of quantification was 4.5 log₁₀ CFU/g, based on a maximum reliably countable plate of 300 colonies in accordance with ISO 7218:2007/Amd.1:2013 (ISO 7218, 2024). Samples with counts below the LOD were reported as <2.0 log₁₀ CFU/g.

Plates exhibiting growth above the quantifiable range (too numerous to count; TNTC) were reported as $>4.5 \log_{10}$ CFU/g.

To identify dominant taxa in the aerobic plate count, isolates with distinct morphologies were randomly selected from countable plates. Selected colonies were re-streaked for purity, resuspended in Hogness' freezing medium (Werner, 1997), and stored at -80°C for Sanger sequencing.

2.4. Sanger sequencing

For analysis, frozen colonies were thawed and centrifuged (Eppendorf 5804, NH, USA) at 13,000 g for 1 min. The supernatant was discarded, and the pellet was resuspended in 1 mL of sterile bacteriological peptone water (NaCl, Merck, 8.5 g/L; Bacteriological peptone, Oxoid, 1 g/L).

DNA extraction, PCR, gel electrophoresis, and Sanger sequencing of the 16S rRNA gene were performed according to Uhlig et al. (2017). DNA extraction was carried out using the DNeasy PowerFood Microbial Kit (QIAGEN GmbH, Hilden, Germany), and the DNA concentration was measured with a NanoDrop spectrophotometer (Thermo Scientific, MA, USA). The purified DNA was used as a template for Polymerase Chain Reaction (PCR) to amplify the 16S rRNA gene.

Primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CTACGGCTACCTTGTACGA-3') (Eurofins Genomics, Ebersberg, Germany), were used with TopTaq DNA Polymerase (Qiagen, Netherlands) and the PCR was performed according to the manufacturers' instructions. PCR products were verified by gel electrophoresis using 1.5% agarose gel (Sigma-Aldrich, Munich, Germany) in Tris Acetate-EDTA (TAE) buffer (Sigma-Aldrich, Munich, Germany). The gel ran at 120 V for 60 min (Biorad PP300, USA) and was stained with GelRed (Biotium, USA) for 30 min.

Successfully amplified PCR products were sent for sequencing at Eurofins Genomics (Ebersberg, Germany) using 27F as the sequencing primer. Sequence similarities were determined by comparing the obtained sequences to NCBI BLASTn databases (Camacho et al., 2009). The Sanger sequencing data and associated metadata have been deposited in Zenodo under accession <https://doi.org/10.5281/zenodo.20700375>.

2.5. Next generation sequencing

DNA from bacterial isolates was extracted using the DNeasy PowerFood Microbial Kit (QIAGEN GmbH, Hilden, Germany). Next generation sequencing (NGS) was performed according to Illumina handbook (Illumina, 2025). The V3–V4 hypervariable region of the 16S rRNA gene was amplified, and the resulting products were purified using AMPure XP beads (Beckman Coulter Genomics, CA, USA). DNA concentrations were measured with a Qubit fluorometer (ThermoFisher Scientific, MA USA) using the dsDNA High Sensitivity Assay. A total of 41 samples, each tagged with unique dual indices, passed quality control and were normalized to 4 nM using Illumina's resuspension buffer. Sequencing was performed on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle) at the Department of Process and Life Science Engineering Laboratory, Lund University, Sweden. PhiX (Illumina, San Diego, USA) was included as an internal control, and a final volume of 600 μL was loaded for sequencing.

Raw sequencing data were analyzed using QIIME2 (version 2025.4), following standard operating procedures. Samples with fewer than 8105 reads were excluded ($n = 1$), and the remaining datasets were rarefied to this depth. Taxonomic classification of operational taxonomic units (OTUs) was conducted using the SILVA database (version 138). The Next Generation sequencing data and associated metadata have been deposited in Zenodo under accession <https://doi.org/10.5281/zenodo.20700375>.

2.6. Phytic acid analysis

Phytic acid was quantified as inositol hexaphosphate (InsP_6) using high-performance ion chromatography (HPIC), following the procedure described by (Carlsson et al., 2001). Approximately 0.5 g of freeze-dried, finely ground PBA's sample was extracted in triplicate with 10 mL of 0.5 mol/L HCl for 3 h on a laboratory shaker (Heidolph Reax 2; Heidolph Instruments, Schwabach, Germany). After extraction, a 1 mL aliquot was withdrawn, centrifuged, and filtered to remove residual fat before being transferred to HPLC vials.

HPIC was carried out using an HPLC pump (PU-4000i; Jasco Inc., Easton, MD, USA) together with an R-HPLC pump (PU-4180; Jasco, Oklahoma City, OK, USA), equipped with a PA-100 guard column and a CarboPac PA-100 analytical column. InsP_6 was eluted isocratically with a mobile phase consisting of 80% 1 mol/L HCl and 20% Milli-Q water at a flow rate of 0.8 mL/min. The eluent underwent post-column derivatization with ferrous nitrate, and detection was performed at 290 nm using a UV-visible detector (UV-4075; Jasco, Oklahoma City, OK, USA). All chromatographic runs were conducted at ambient temperature. Each sample was analyzed for 7 min, and InsP_6 concentrations were determined using external calibration standards in the range of 0.1–0.6 $\mu\text{mol/L}$.

2.7. Iron analysis

Iron concentration was analyzed in duplicate using atomic absorption spectrometry (200 Series AA Systems; Agilent). Before measurement, samples were digested under acidic conditions in a microwave digestion system (EthosPlus; Milestone Microwave Laboratory Systems, Sorisole, Italy), following the protocol described by Mayer Labba et al. (2022a). Calibration curves were generated using commercial standard solutions with a concentration range of 0.125–5.0 mg/L. The method's limit of detection (LOD) was 0.1 mg/L.

2.8. Estimated bioavailability of iron

Iron bioavailability was estimated based on the approach previously described by Mayer Labba et al., (2022a) and can be used as an indication of the inhibitory effect of phytic acid on iron. The molar ratio of phytic acid to iron (PA:Fe) was calculated to estimate iron absorption as phytic acid can act as an inhibitor. The molecular weights of 660.3 g/mol for phytic acid and 55.845 g/mol for iron were used in the calculations.

2.9. Statistical analysis

To identify the closest type of strains from Sanger sequencing data, the Basic Local Alignment Search Tool (BLASTn) was used. Graphs based on Sanger and NGS data were created using RStudio with the tidy and ggplot2 packages.

Adapter and primer sequences were trimmed using cutadapt, and sequence data were processed using DADA2 to identify amplicon sequence variants (ASVs). ASVs were aligned using MAFFT, and a phylogenetic tree was constructed using FastTree2. Taxonomic and compositional comparisons presented in this study were descriptive; no inferential statistical comparisons of 16S community composition were performed.

The results from phytic acid and iron analysis, as well as iron bioavailability, were expressed as the mean value with its corresponding standard deviation, and each analysis was conducted in triplicate for the phytic acid measurements and in duplicate for the iron content analysis. Data were assessed for normality using the Shapiro–Wilk test. When data did not meet the assumptions of normality, the non-parametric Kruskal–Wallis test was applied. In cases where normality was satisfied, comparison of means was conducted using one-way analysis of variance (ANOVA), according to Tukey's test, with a significance level of

$\alpha = 0.05$. The statistical analysis was performed with the statistical software IBM SPSS 29 (IBM, New York, U.S.A.)

3. Results

3.1. Microbial enumeration and isolation

Of the 40 PBAs, 25 products had total aerobic counts (TSA plates) below $2.0 \log_{10}$ CFU/g, 7 had counts within the quantifiable range, and 8 exceeded $4.5 \log_{10}$ CFU/g (Table 2). The corresponding numbers for lactic acid bacteria (MRS plates) were 34, 4, and 2, respectively, while 6 products had quantifiable yeast and mold counts on MA plates. *Enterobacteriaceae* counts on VRBD plates were below the detection limit of $2.0 \log_{10}$ CFU/g for all products. Quantifiable counts and TNTC occurred predominantly among meat alternatives, whereas dairy alternatives generally showed lower counts across all media evaluated. Within the meat alternatives, higher aerobic counts were observed particularly among formulated heat-treated products, although substantial variation was evident between products within the same processing category. Following heat activation, *Bacillus* spp. were recovered on BCA plates from 28 products after 6 h and 37 products after 16 h.

3.2. Sanger sequencing

A total of 120 isolates were identified by Sanger sequencing. The identities of the 16S rRNA gene-sequenced isolates recovered from PBAs and the cow's milk reference sample are provided in Supplementary Data, Table S1 and Table S2.

Sanger sequencing revealed a diverse range of both spore-forming and non-spore-forming genera. As shown in Fig. 1A, dairy analogues contained a higher proportion of spore-forming isolates (70.0%) compared with meat analogues (49.0%). In contrast, non-spore-forming isolates were slightly more prevalent in meat analogues. These results indicate that dairy analogues contained a higher percentage of spore-forming bacteria, whereas non-spore-forming bacteria were slightly more dominant in meat analogues.

The number of bacterial species identified within each genus indicated greater microbial diversity in meat analogues than in dairy analogues (Fig. 1B). Meat analogues showed greater diversity within *Bacillus*, *Lactobacillus*, *Brevibacillus*, and *Pseudomonas*, whereas dairy analogues were predominantly associated with *Staphylococcus*, *Oceanobacillus*, *Brevibacillus*, *Pseudobacillus*, and *Bacillus*.

The distribution of genera across ingredient sources and product categories is shown in Fig. 1C. Several genera exhibited ingredient-specific distributions, with LAB-associated genera primarily detected in pea- and soy-based PBAs, while spore-forming genera were more

Table 2

Viable counts of total aerobic counts, *Enterobacteriaceae*, yeasts and molds, lactic acid bacteria, and spore-forming *Bacillus* spp. in PBAs from the Swedish market, expressed as mean $\log_{10}$ CFU/g $\pm$ SD of six replicates. Total aerobic counts were enumerated on TSA, *Enterobacteriaceae* on VRBD, yeasts and molds on malt extract agar, lactic acid bacteria on MRS, and spore-forming *Bacillus* spp. on BCA. The limit of detection was $2.0 \log_{10}$ CFU/g for all media.

Product category	Sample code	Lactic Acid Bacteria ($\log_{10}$ CFU/g $\pm$ SD)	Total Aerobic Count ($\log_{10}$ CFU/g $\pm$ SD)	<i>Enterobacteriaceae</i> ($\log_{10}$ CFU/g $\pm$ SD)	Yeasts and molds ($\log_{10}$ CFU/g $\pm$ SD)	<i>Bacillus</i> spp. 16h 60°C ($\log_{10}$ CFU/g $\pm$ SD)	<i>Bacillus</i> spp. 6h 60°C ($\log_{10}$ CFU/g $\pm$ SD)
Meat analogue	MA1	<2.0	>4.5	<2.0	2.68 ± 0.00	<2.0	<2.0
	MA2	<2.0	3.42 ± 0.00	<2.0	2.99 ± 0.00	>4.5	<2.0
	MA3	<2.0	2.81 ± 0.00	<2.0	<2.0	<2.0	<2.0
	MA4	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA5	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA6	<2.0	2.67 ± 0.00	<2.0	<2.0	<2.0	<2.0
	MA7	2.78 ± 0.00	2.92 ± 0.00	<2.0	3.04 ± 0.00	<2.0	<2.0
	MA8	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA9	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA10	>4.5	>4.5	<2.0	<2.0	<2.0	<2.0
	MA11	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA12	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA13	>4.5	>4.5	<2.0	3.64 ± 0.00	<2.0	<2.0
	MA14	<2.0	>4.5	<2.0	>4.5	<2.0	<2.0
	MA15	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA16	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA17	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA18	<2.0	<2.0	<2.0	2.81 ± 0.00	<2.0	3.14 ± 0.00
	MA19	4.56 ± 0.00	>4.5	<2.0	<2.0	<2.0	<2.0
	MA20	2.57 ± 0.00	>4.5	<2.0	<2.0	<2.0	>4.5
	MA21	<2.0	<2.0	<2.0	<2.0	<2.0	>4.5
	MA22	<2.0	<2.0	<2.0	<2.0	<2.0	>4.5
	MA23	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA24	<2.0	2.67 ± 0.00	<2.0	<2.0	<2.0	<2.0
	MA25	<2.0	<2.0	<2.0	<2.0	<2.0	>4.5
	MA26	<2.0	>4.5	<2.0	2.78 ± 0.00	<2.0	>4.5
	MA27	<2.0	2.04 ± 0.00	<2.0	<2.0	<2.0	>4.5
	MA28	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA29	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	MA30	2.24 ± 0.00	>4.5	<2.0	>4.5	<2.0	2.52 ± 0.00
	MA31	<2.0	2.38 ± 0.00	<2.0	<2.0	>4.5	<2.0
	MA32	<2.0	<2.0	<2.0	<2.0	<2.0	>4.5
	MA33	<2.0	<2.0	<2.0	<2.0	<2.0	2.15 ± 0.00
	MA41	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Dairy analogue	DA34	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	DA35	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	DA36	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	DA37	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
	DA38	<2.0	<2.0	<2.0	<2.0	<2.0	>4.5
	DA39	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0
Cow's milk	SM40	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0

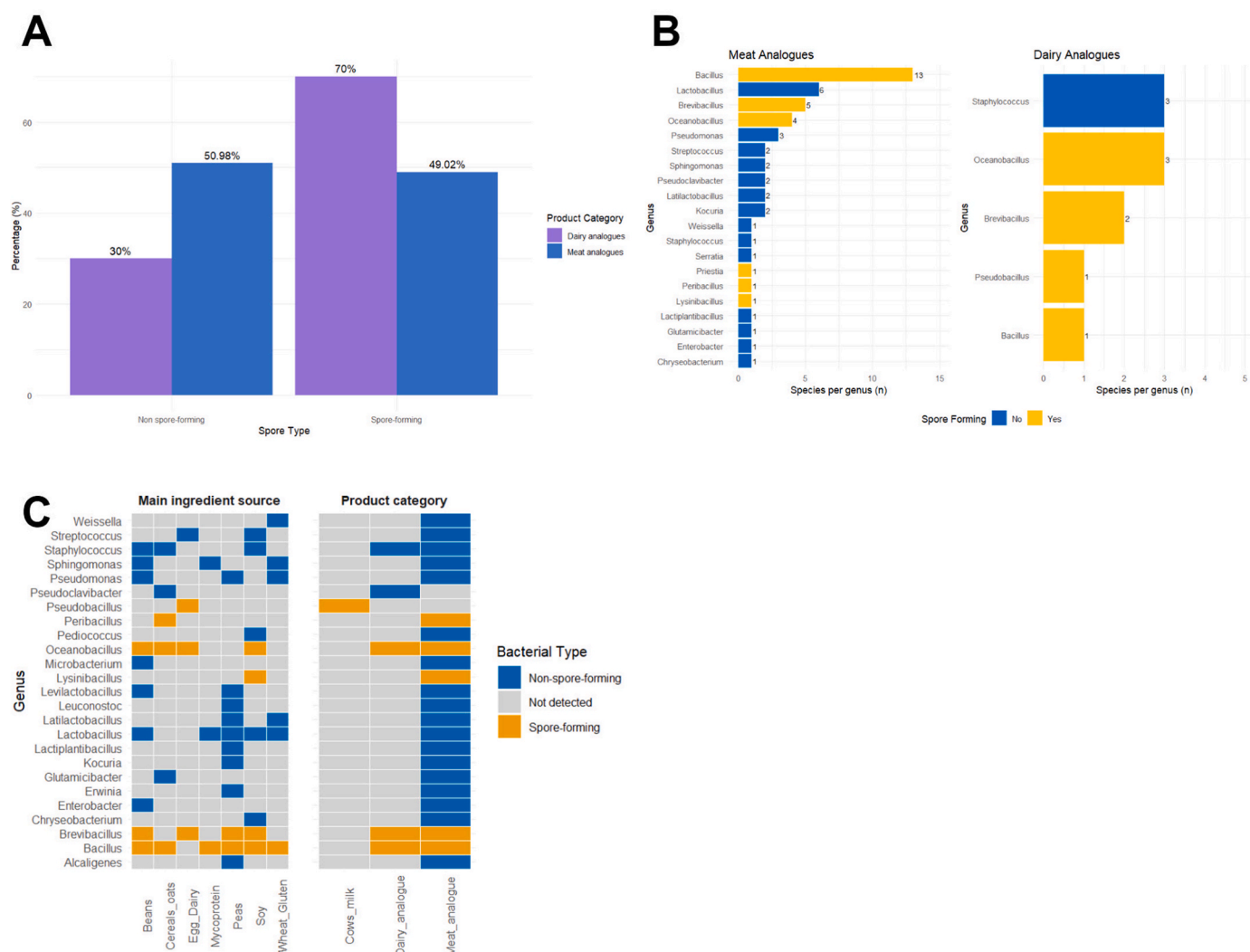


Fig. 1. Results from the Sanger sequencing.

(A) Total percentage of spore-forming and non-spore-forming bacterial genus identified in PBAs. All isolates identified by Sanger sequencing were included in the analysis. Detailed species identities are provided in Supplementary Data, [Table S1 & S2](#).

(B) The total number of bacterial species per genera identified in PBAs. All isolates identified by Sanger sequencing were included in the analysis. Detailed species identities are provided in Supplementary Data, [Table S1 & S2](#).

(C) Heatmap of spore-forming and non-spore-forming genera identified in PBAs, grouped by main ingredient source and product category. Color indicates the presence or absence of each genus in the respective group. All genera were identified by Sanger sequencing. In the Egg/Dairy group, genera detected in both standard cow's milk (SM40) and vegetarian sausage A (MA6) are included.

prevalent in soy- and cereal-based products.

3.3. Distribution of *Bacillus* spp.

Several *Bacillus* species were identified in both meat and dairy analogues before and after spore activation (Fig. 2A). A greater number of *Bacillus* spp. were detected after 16 h of heat activation compared with non-activated samples. *Bacillus velezensis* and *Bacillus sporothermodurans* were detected both before and after heat activation.

The distribution of *Bacillus* spp. across storage conditions is shown in Fig. 2B. Prior to spore activation, viable *Bacillus* spp. were detected in both refrigerated and frozen products. Following 6 and 16 h of heat activation, most *Bacillus* spp. were recovered from frozen products. *Bacillus cereus* was detected only after spore activation in refrigerated and room-temperature products, whereas *B. zhangzhouensis*, *B. thuringiensis*, *B. pumilus*, and *B. licheniformis* were detected only after spore activation in refrigerated and frozen products.

The distribution of spore-forming genera according to processing category is shown in Fig. 2C. Formulated heat-treated products

exhibited the highest number of spore-forming isolates, with *Bacillus* as the dominant genus, followed by *Brevibacillus*, *Oceanobacillus*, and *Lysinibacillus*. Beverages also exhibited considerable diversity of spore-forming genera. Across all processing categories, *Bacillus* was the predominant genus.

3.4. Next generation sequencing

Next-generation sequencing (NGS) profiles were compared descriptively across product and ingredient categories (Fig. 2D). NGS revealed a broader range of bacterial genera than Sanger sequencing, particularly among non-spore-forming bacteria. Consistent with the Sanger sequencing results, meat analogues exhibited greater bacterial diversity than dairy analogues and the cow's milk reference sample, although no inferential statistical comparison of community diversity was performed.

Several genera were shared across product categories, whereas others exhibited ingredient-specific distributions. *Xanthomonas* was detected only in pea- and oat-based meat analogues, while *Periweissella*,

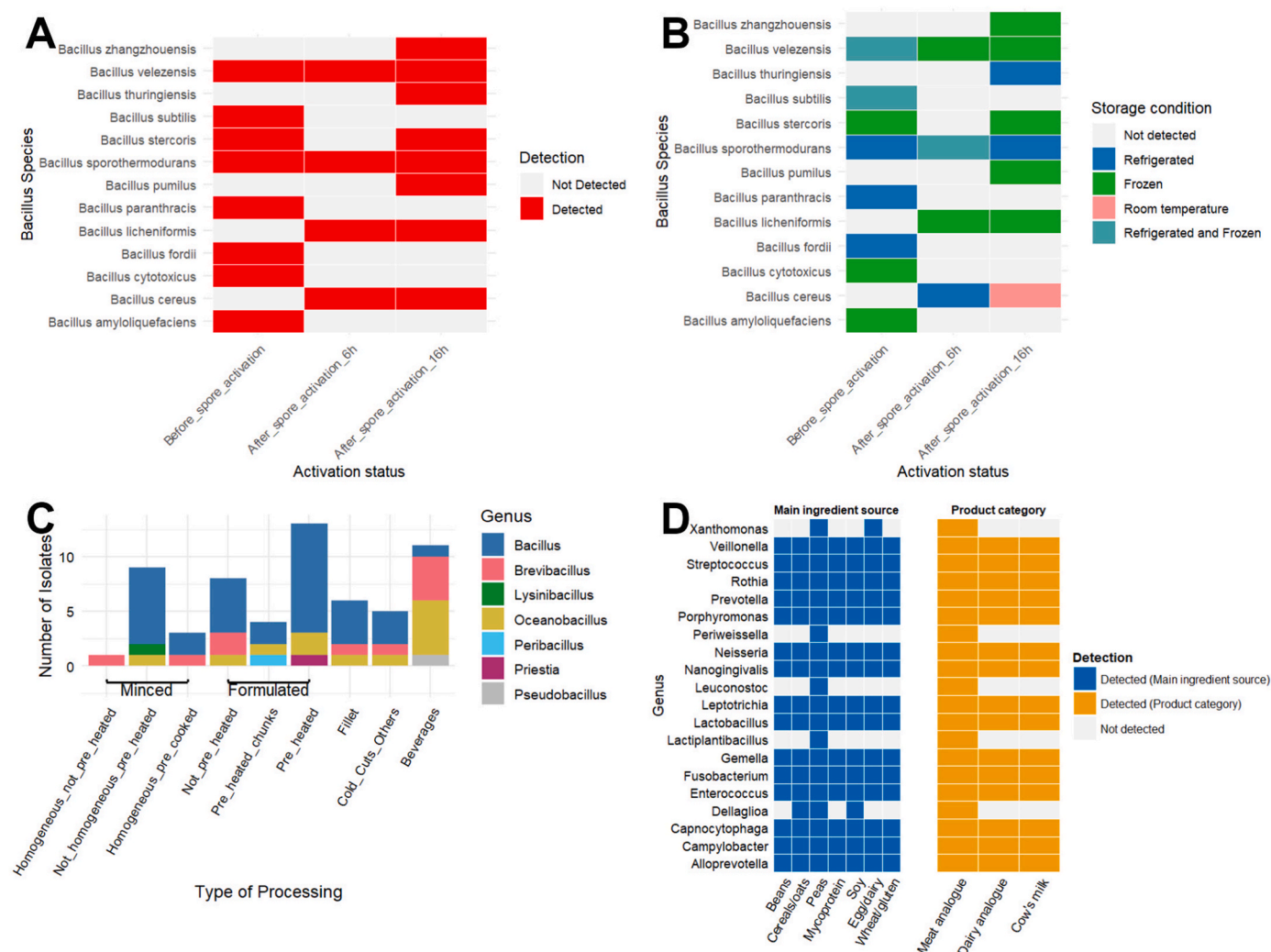


Fig. 2. Distribution of spore-forming bacteria and bacterial community composition in plant-based alternatives (PBAs).

(A) Heatmap showing *Bacillus* species identified in meat and dairy analogues before and after spore activation. Isolates were recovered from Malt Extract Agar (MA), Tryptic Soy Agar (TSA), De Man, Rogosa and Sharpe Agar (MRS), and Bacillus ChromoSelect Agar (BCA) following incubation at 30°C for 48 h, and after heat treatment at 60°C for 6 h and 16 h. Color indicates the presence or absence of each species.

(B) Heatmap showing the distribution of *Bacillus* genera according to storage condition (refrigerated, frozen, and room temperature). Genera were identified by Sanger sequencing. "Refrigerated and Frozen" indicates species detected in both storage conditions.

(C) Distribution of spore-forming bacterial genera identified by Sanger sequencing according to processing types. Bars represent the number of isolates identified for each genus across the different processing categories. Detailed species identities are provided in Supplementary Table S1 [Appendix A](#).

(D) Heatmap of the 20 most abundant bacterial genera identified by NGS, grouped according to main ingredient source and product category. Color indicates the presence or absence of each genus. The Egg/Dairy category includes both the conventional cow's milk reference sample (SM40) and the vegetarian sausage sample (MA6).

Leuconostoc, and *Lactiplantibacillus* were detected primarily in pea-based meat analogues. *Dellagloba* was associated with oat-, pea-, and soy-based meat analogues.

Among the 20 most abundant genera identified by NGS were several genera commonly associated with human oral and gut microbiota, including *Campylobacter*, *Prevotella*, *Rothia*, *Porphyromonas*, *Neisseria*, and *Lautropia*.

3.5. Phytic acid content

Phytic acid content varied substantially among the products investigated ([Fig. 3A](#)). However, when grouped according to processing type, no significant differences were observed among groups, except for the beverages group which showed significant lower values ($p > 0.05$).

In contrast, grouping according to main ingredient source revealed significant differences in phytic acid content ([Fig. 3B](#)). Products based

on nuts, oats, egg/dairy, and wheat/gluten exhibited the lowest phytic acid levels, whereas soy-, bean-, mycoprotein-, peas- and mixed-legume-based products generally exhibited higher levels.

The reference sample, which was cow's milk, showed a signal below the limit of detection (LOD).

3.6. Iron content

No significant differences in iron content were observed among processing categories ([Fig. 3C](#)). According to the labels of two samples (MA15, MA16), iron oxide had been added as a colorant, which may have interfered with the analysis resulting in higher values. For this reason, they were excluded from [Fig. 3C & D](#). Both samples belonged to the cold-cut category and were classified as mycoprotein-based products. Since the mycoprotein group contained only these two samples, the group was excluded from [Fig. 3D](#).

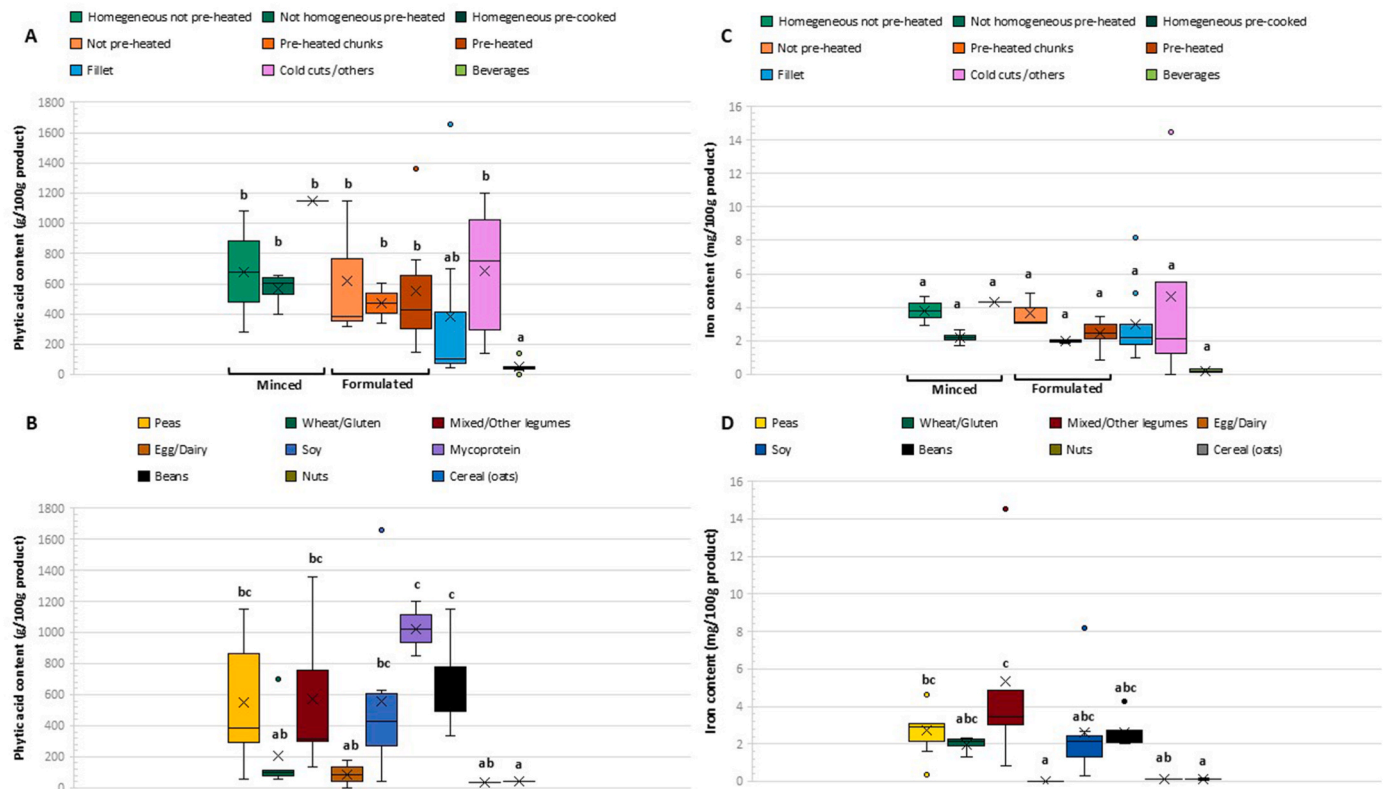


Fig. 3. Phytic acid- and Iron content of PBA's, expressed as mg per 100 g of product.

(A) Phytic acid content of samples grouped by processing type.

(B) Phytic acid content of samples grouped by main ingredient source. Different letters indicate statistically significant differences among samples ($p < 0.05$).

(C) Iron content of samples grouped by processing type.

(D) Iron content of samples grouped by main ingredient source. Different letters indicate statistically significant differences among samples ($p < 0.05$).

When grouped according to ingredient source, significant differences in iron content were observed (Fig. 3D). Egg/dairy-, and oat-based products exhibited the lowest iron content which is expected as eggs and dairy products contain minimal iron. In contrast, pea-based products exhibited significantly higher iron levels. Mixed-legume-based products showed the highest overall iron content.

3.7. Estimated bioavailability of iron

Quantifying the iron content alone does not adequately represent a product's nutritional value, it is also crucial to consider the mineral's bioavailability. Hence Fig. 4 represents the molar ratio between phytic acid and iron (PA:Fe) which can be used as a theoretical indicator of how much the present phytic acid can chelate iron and hence inhibit iron absorption.

The phytic acid-to-iron molar ratio (PA:Fe) varied substantially among products. One sample exhibited PA:Fe ratio below 1 (MA4), while six additional samples had ratios below 6 (MA9, MA11, MA12, MA18, MA22, MA26). Most of those samples contained wheat (gluten) as the primary ingredient, while the others were based on soy, peas or other legumes. All samples except one belonged to the fillet category.

Significant differences were observed between processing categories, with beverages exhibiting the highest PA:Fe ratios and fillets products exhibiting the lowest ratios (Fig. 4A).

When grouped according to main ingredient source, cereal-based products exhibited the highest PA:Fe ratios, whereas wheat/gluten-based products exhibited the lowest ratios (Fig. 4B).

4. Discussion

This study explored the microbiological and antinutritional risks associated with the consumption of plant-based meat and dairy alternatives (PBAs) in comparison with cow's milk. Culture-dependent methods, Sanger sequencing, and next-generation sequencing (NGS) were used to characterize microbial composition, while phytic acid content and estimated iron bioavailability were used to assess potential antinutritional risks. Plant-based foods typically contain diverse microbial communities and varying levels of phytic acid, which may reduce mineral bioavailability. Overall, substantial variation was observed among individual PBAs, both in microbial counts and microbial composition, indicating that these products cannot be considered a microbiologically homogeneous food category. Ingredient source and processing type also appeared to influence microbial patterns, while the main ingredient source had a stronger influence than processing category on phytic acid and iron content.

Unlike conventional dairy and meat products, PBAs are not standardized food categories under current EU Regulation (EC) No. 2073/2005, and microbiological criteria established for animal-derived foods may therefore not be directly applicable to these products (European Parliament and Council, 2004; Kabisch et al., 2024). This is particularly relevant considering the large variation observed in the culture-based analyses. While many products had microbial counts below the detection limit ($<2.0 \log_{10}$ CFU/g), others showed quantifiable counts or exceeded the upper quantification limit ($>4.5 \log_{10}$ CFU/g). Higher culture-based counts occurred predominantly among meat alternatives, whereas most dairy alternatives showed counts below the detection limit. However, substantial variability was also evident within the meat-alternative group, suggesting that

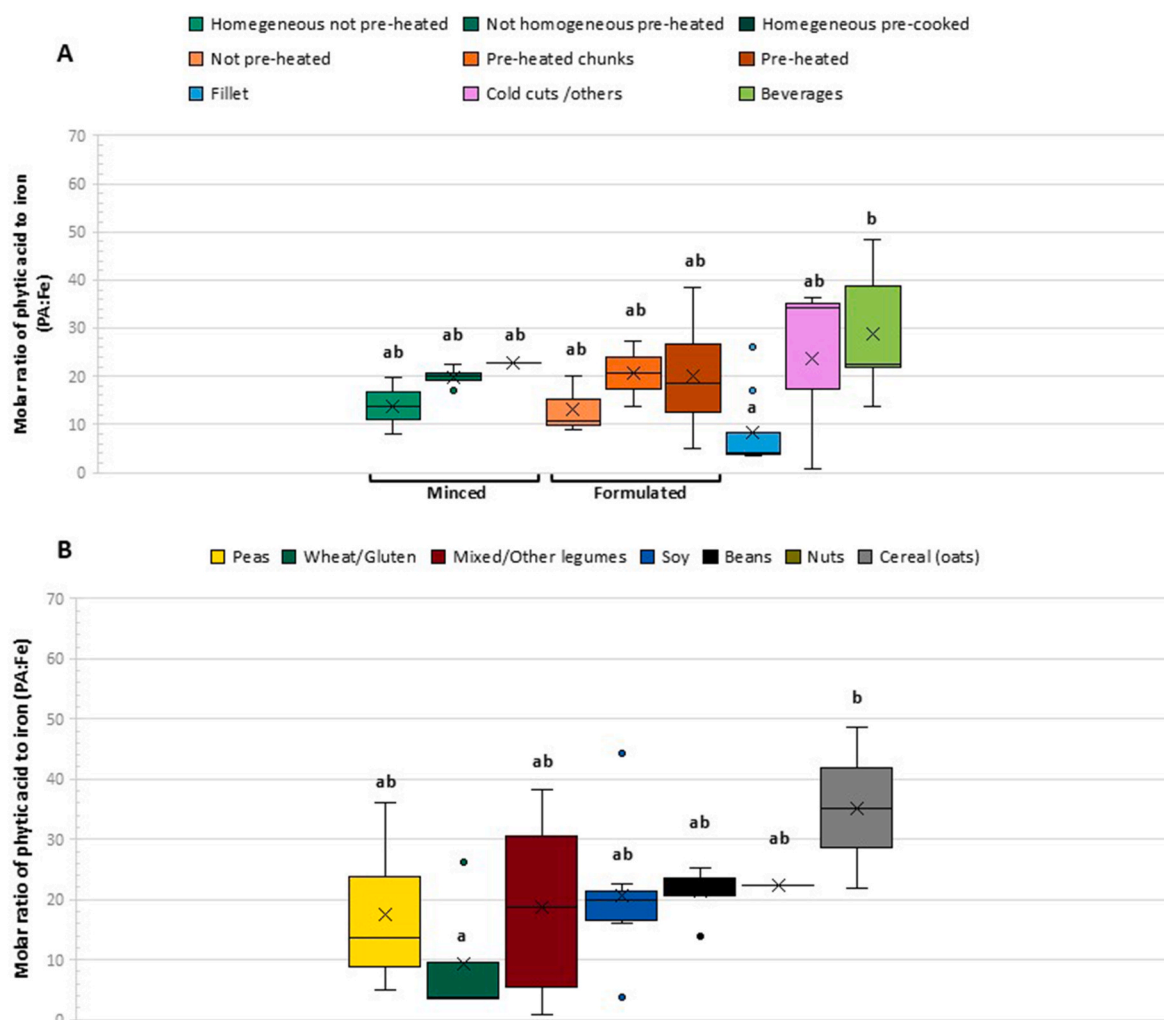


Fig. 4. The molar ratio of phytic acid to iron (PA:Fe) of PBA's.

(A) Samples grouped by processing type.

(B) Samples grouped by main ingredient source. Different letters indicate statistically significant differences among samples ($p < 0.05$).

microbial load may depend on product-specific characteristics rather than product category alone (Table 2).

Differences in processing may partly explain this variability. PBAs with microbial counts above the quantifiable range were not restricted to a single processing category, although such values were more frequently observed among formulated heat-treated meat alternatives (Fig. 2C). These products commonly undergo several processing steps and may contain multiple ingredients added after extrusion, including oils, stabilizers, and flavoring agents (Saerens et al., 2021; Schmid et al., 2022). Consequently, high counts in some formulated products may reflect microorganisms associated with individual ingredients, survival through processing, or introduction during post-processing handling. In contrast, several products within the same processing categories had microbial counts below the detection limit, demonstrating that processing type alone does not explain the observed variation. Because the number of samples within individual processing categories was relatively small and uneven, these observations should be regarded as descriptive trends rather than statistically established associations.

A similar product-specific pattern was observed for spore-forming bacteria. *Bacillus* was the predominant spore-forming genus and was detected across several ingredient and processing categories, although its occurrence and diversity varied considerably among products. Soy-, bean-, and cereal/oat-based PBAs were more frequently associated with spore-forming genera, and soy-based products contained the highest

diversity of spore-formers (Fig. 1C). Formulated heat-treated products also exhibited greater diversity of spore-forming bacteria than non-homogeneous minced products (Fig. 2C). These patterns may partly reflect differences in the microbiota of the main ingredient source as well as processing history. Plant-derived ingredients can introduce spores from the agricultural environment, while heat-resistant spores may survive processing steps that eliminate vegetative microorganisms. Post-processing addition of ingredients may provide an additional route for microbial introduction.

The heat-activation experiments further demonstrated differences in the recovery of *Bacillus* spp. Several species were detected only following exposure to 60°C, and some were recovered only after prolonged heat activation (Fig. 2A). Notably, *B. cereus* was detected only following spore activation, while *B. velezensis* and *B. sporothermodurans* were detected both before and after activation. These findings suggest that at least some spores were present in a dormant state and required suitable activation conditions to become detectable under the experimental conditions. Previous studies have shown that dormant *Bacillus* spores can survive thermal processing and subsequently germinate under favorable conditions (Berendsen et al., 2016; Setlow, 2006). *B. sporothermodurans*, for example, is known for producing highly heat-resistant spores capable of surviving ultra-high-temperature processing (Scheldeman et al., 2006), while *B. velezensis* has been associated with stress tolerance and biofilm formation (Xie et al., 2024).

The Swedish Food Administration (Livsmedelsverket), in accordance with Regulation (EC) No. 852/2004, recommends warm holding above 60°C to maintain food safety margins for pathogenic bacteria (European Parliament and Council, 2004; Livsmedelsverket, 2025). The recovery of *B. cereus* following heat activation at this temperature does not demonstrate growth during warm holding, but it indicates that exposure to 60°C may not necessarily eliminate dormant spores in all plant-based matrices. The present study did not assess germination, vegetative growth, or toxin production during actual cooking or warm-holding conditions. Therefore, further studies under realistic food-handling conditions are needed before conclusions regarding consumer risk can be drawn. Nevertheless, the results demonstrate that conventional enumeration without an activation step may not recover all viable spore-forming bacteria present in PBAs.

Storage conditions may also contribute to the observed variability (Fig. 2B). Viable *Bacillus* spp. were detected in both refrigerated and frozen products prior to heat activation. Freezing can preserve bacterial spores in a dormant but viable state (Nečas & Gabriel, 1978), and although low temperatures inhibit vegetative growth, they do not necessarily eliminate spores. Most *Bacillus* species recovered following heat activation were associated with refrigerated or frozen products, further demonstrating their persistence across different storage conditions. However, because storage conditions were related to product type and the number of products within each storage category differed, the effects of storage temperature cannot be separated from those of formulation and processing in the present study.

Differences between culture media and sequencing approaches also affected microbial recovery. Although *Bacillus* ChromoSelect Agar (BCA) was used specifically for *Bacillus* enumeration, some *Bacillus* species were recovered on MA, MRS, and TSA before heat activation (Supplementary Data, Table S1 and Table S2). These media were not used for the subsequent spore-activation enumerations, which may partly explain why some species identified before activation were not subsequently recovered. This demonstrates that culture-medium selection can substantially influence the microorganisms recovered and should be considered when designing microbiological quality-control protocols for PBAs.

Both Sanger sequencing and NGS revealed frequent occurrence of lactic acid bacteria (LAB), particularly in pea-, bean-, and soy-based products (Fig. 2D). Species including *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Lactobacillus sakei*, and *Pediococcus acidilactici* were identified, consistent with previous studies reporting LAB as common members of the microbiota of plant-based meat alternatives (Duthoo et al., 2022; Geeraerts et al., 2020; Kabisch et al., 2024). Although LAB are generally not associated with foodborne illness, they can contribute to spoilage through souring, gas formation, and slime production during storage.

Sequencing also detected taxa of potential hygienic relevance. *Enterococcus* was identified by NGS, in all ingredient types (Fig. 2D). While some members of this genus are naturally associated with plants, others are opportunistic human pathogens, and their presence in foods may reflect microorganisms originating from raw materials or introduced during processing (Geeraerts et al., 2020). NGS additionally detected *Campylobacter*, *Prevotella*, *Rothia*, *Porphyromonas*, *Neisseria*, and *Lautropia*, genera commonly associated with human oral or gut microbiota. However, detection of bacterial DNA by NGS does not demonstrate the presence of viable organisms or pathogenic strains. These findings should therefore be interpreted cautiously and may represent low-level environmental or handling-associated contamination. Further viability-based analyses would be required to establish their relevance to food safety. It nevertheless emphasizes the importance of hygienic handling and processing practices, particularly for products marketed as ready-to-eat foods.

Regarding the nutritional analyses, substantial variation in phytic acid content was observed among PBAs, with ingredient source exerting a greater influence than processing category (Fig. 3A & B). Products

belonging to the egg/dairy group exhibited the lowest phytic acid levels, which is expected given the absence of plant-derived ingredients naturally rich in phytic acid. Nuts- and oat-based products also showed relatively low phytic acid levels, although previous studies have reported conflicting findings regarding the phytic acid content of oats and oat-based products (Alemayehu et al., 2022; Zhang et al., 2007).

Pea-based products exhibited high phytic acid levels, consistent with previous reports indicating that extrusion processing may have limited effects on phytic acid content in peas (Nasrollahzadeh et al., 2022). Similar findings have previously been reported by Mayer Labba et al., (2022a), who demonstrated that phytic acid content in plant-based meat analogues was influenced by both the main protein source and protein extraction method. In the present study, mycoprotein-, soy-, bean-, and mixed-legume-based products generally exhibited elevated phytic acid levels, consistent with earlier observations for soy protein and legume-derived ingredients (Mayer Labba et al., 2022b). Considerable variability was observed within several ingredient groups, which may partly reflect differences in raw materials, formulation, and processing conditions. Previous studies have shown that processing methods such as protein extraction and hydrothermal treatment can substantially influence phytic acid content (Deak & Johnson, 2007; Fredrikson et al., 2001; Helstad et al., 2022). However, detailed information regarding industrial processing conditions was not available for the investigated commercial products, limiting further interpretation of these findings.

Iron content also varied considerably among the products investigated (Fig. 3C & D). Pea- and mixed-legume-based products generally exhibited higher iron contents, whereas nut-, oat-based products and products containing mainly eggs and dairy showed lower values. Since the investigated PBAs were plant-derived, the detected iron consisted primarily of non-heme iron. Compared with heme iron, non-heme iron generally exhibits lower bioavailability and is more strongly influenced by dietary inhibitors such as phytic acid, polyphenols, and calcium, although absorption may be enhanced by components such as vitamin C (R. R. Hurrell & Egli, 2010).

The nutritional relevance of iron content should therefore be interpreted together with iron bioavailability. The phytic acid-to-iron molar ratio (PA:Fe) is commonly used as an indicator of the inhibitory effect of phytic acid on iron absorption, with lower ratios corresponding to higher estimated bioavailability (Hallberg et al., 1989; Siegenberg et al., 1991). More specifically, the molar ratio should be below 1, ideally under 0.4, to significantly improve iron absorption (R. R. Hurrell & Egli, 2010). When vitamin C and meat or fish are consumed at the same meal, the PA:Fe molar ratio should not be higher than 6 (Dahdouh et al., 2019; R. R. Hurrell & Egli, 2010). In the present study, it was noticed that only one sample had a value below 1 and only a few below 6. Most products displayed elevated PA:Fe ratios, suggesting low estimated iron bioavailability (Fig. 4A & B). The overall high phytic acid-to-iron ratios in the investigated food products imply a potential risk of low iron absorption in the gastrointestinal tract, as phytic acid can chelate iron and thereby hinder its uptake. It is also important to mention that relying only on these products to achieve the daily iron requirements is difficult due to their low estimated bioavailability. These findings are relevant given the increasing replacement of animal-derived foods with PBAs and the growing concern regarding iron deficiency among populations consuming plant-based diets.

Several processing strategies, including hydrothermal treatment, phytase treatment, and fermentation, have previously been shown to reduce phytic acid levels and improve mineral bioavailability (Bergman et al., 1999; Fredlund et al., 1997; R. F. Hurrell et al., 2003; Kasaoka et al., 1997; Pongrac et al., 2016). Such approaches may therefore be relevant for improving the nutritional quality of PBAs.

5. Conclusion

Overall, spore-forming bacteria represent an important

consideration for the microbiological quality and safety of plant-based alternative products. These microorganisms can survive standard food processing conditions and may remain dormant until conditions favor germination. Extended heat activation appeared important for detecting a broader range of spore-formers in these products. Soy-, cereal-, and bean-based PBAs were more frequently associated with spore-forming genera, highlighting the relevance of ingredient-specific microbiological monitoring and processing strategies.

In addition to microbiological considerations, most PBAs exhibited relatively high phytic acid levels and high phytic acid-to-iron molar ratios, indicating low estimated iron bioavailability. These findings emphasize the importance of evaluating not only iron content but also mineral bioavailability in PBAs, particularly as these foods increasingly replace animal-derived products with high mineral bioavailability in human diets.

Further studies are needed to evaluate the viability and functional relevance of detected microorganisms, as well as the implications of these findings for food safety and nutrition.

6. Study limitations

Several limitations should be considered when interpreting these findings. The number of products within some ingredient, processing, and storage categories was small and uneven, limiting statistical comparisons of microbiological counts among groups. In addition, a substantial proportion of the culture-based measurements fell either below the detection limit or above the upper quantification limit, restricting direct quantitative comparisons among individual products. Sequencing-based detection identifies microbial DNA but does not confirm viability, pathogenicity, or toxin production. Furthermore, conventional meat products were not included, hence direct comparisons with plant-based meat alternatives could not be made. Instead, the study focused on including as many different plant-based products on the market as possible. This allowed for the screening of a broad range of products and provided an overall picture of the market without limiting the selection to products with comparable conventional meat products. Future studies using larger and more balanced product groups, quantitative microbiological approaches, viability and toxin analyses, and direct assessments of mineral bioavailability are therefore needed to further evaluate the microbiological and nutritional risks associated with PBAs.

CRediT authorship contribution statement

Rumathi de Mel: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Evangelia Papagianni:** Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation, Formal analysis. **Inger-Cecilia Mayer Labba:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis. **Karolina Östbring:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Jeanette Purhagen:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Åsa Håkansson:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2026.120018>.

Data availability

The link to Sanger sequencing data and associated metadata generated during this study can be found at [Attach File step](#). All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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