Plant-based meat alternatives and their associated microbial communities

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Abstract

This is the abstract. It consists of two paragraphs.

Keywords: keyword1, keyword2

1. Introduction:

For most of the population of the Western world, meat consumption is an integral part of their diet. An average U.S. American consumed 100kg and a European Union citizen 69kg of meat in 2021. The global meat consumption increased from 21kg per year and capita in 1990 to 34kg in 2021 (OECD data). Although the OECD estimates that consumption will level off at around 35kg per year and capita by 2030, the total meat consumption will further increase with population growth. This globally growing appetite for meat is linked to livestock farming and consequently plays a major role in the ecological issues we are currently facing. Land degradation, climate change, water pollution and biodiversity loss are just some of the notable consequences for the environment (Steinfeld, 2006; Bianchi et al., 2018). Additionally, common industrial animal husbandry influences public health by supporting the spread of antibiotic resistances and vector-borne disease (Economou and Gousia, 2015; Bianchi et al., 2018; Watts et al., 2018). Apart from this, high meat consumption, as practiced in the Western world and increasingly in transition countries, contributes significantly to many widespread common diseases, which, in addition to individual illnesses, also burden health care systems (Micha et al., 2010; Chan et al., 2011; Parkin et al., 2011; Feskens et al., 2013). Animal welfare, environmental issues and increased health awareness are main drivers for more and more people in western civilization to change their meat consumption routines (Stoll-Kleemann and Schmidt (2017); Ploll and Stern (2020)]. In a survey, conducted 2021 in ten different European countries, 2% of the participants referred to themselves as vegan, 5% as vegetarians, 3% as pescetarians and 30% as flexitarians (European

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Union's Horizon 2020 reasearch and innovation programme, b). The last group is characterized, among other things, by the fact that they want to reduce their meat consumption, but do not want to give up the positive experience that comes with it. As main target group, flexitarians account for 90-95% of the sales of vegan and vegetarian products. A market research within "The Smart Protein Project" noted a sales value increase of 82% for plant-based meat (vegan and vegetarian) within 2018 and 2020 for Austria (European Union's Horizon 2020 reasearch and innovation programme, a). The sales of this product group and the number of different products have increased strongly over the last few years (Curtain and Grafenauer, 2019; European Union's Horizon 2020 reasearch and innovation programme, a). Despite the increased consumption of these products, there have been only a few studies on their microbiological properties. However, these would be important for ensuring food safety, characterizing potential hazards, assessing risks, and for sustainability questions. Since two of the UN's sustainability goals (goal 2 – zero hunger and 12 – responsible consumptions and production) affects our eating habits, increased attention should be paid to reducing food waste. As 30% of food products in primary processing does not even reach the consumer, mainly because of microbial spoilage or pathogen contamination, it is essential to improve the knowledge of the microbial communities of our food, which could help to increase the shelf life and reduce the contamination with pathogens. Still largely unanswered is the question if and how a high microbial diversity on food (consisting of living and dead microbiota) can have a positive effect on consumer's health. For example, a high microbial diversity permanently stimulates the innate and adaptive immune system and provides resistance against colonizing pathogens (Crowe et al., 1973, Mackowiak (1982), Smith et al. (2007)). It was also supposed recently, that the loss of microbial diversity including the disappearance of ancestral indigenous microbiota, which is currently happening in western countries, affects human health and contributes to post-modern conditions such as obesity and asthma (Blaser and Falkow, 2009; Vangay et al., 2018). For all these research questions fundamental knowledge on the microbial compositions on food are necessary, but still lacking. Since, little is known on these products, we sampled a selection of plant-based meat alternatives available in Austria's supermarkets to investigate the general microbial community patterns. Further we described characteristic microbial profiles and compared four groups of the most common product types (pea and soybean based products with either "minced" (minced meat, burgers, etc.) or "fibrous" (meat chunks, Schnitzel, etc.) texture. We hypothesized that products within one group have, based on similar protein processing, more similar communities than between the groups. Since we examined a relatively undescribed product group, we have chosen a combined approach using culture-dependent and culture-independent techniques. This allowed a more complete description of the microbial community than any of the applications could stand

on their own, but also raised additional questions and uncertainties that are relevant to future microbial research of (highly processed) foods.

2. Material and Methods

2.1. Sample acquisition

We purchased 32 different plant-based meat alternative products, between July 12 and July 14, 2021, from four supermarket groups. Pea- and soybean- protein based products with either a minced or a fibrous structure were the most common representatives of this product group at this time point in Austria. Additional criteria for the selection were, that the products where entirely plant-based (vegan) and do not contain fermented products, like tofu. Beside these characteristics, the samples were different in their composition, packing, shelf-life etc. (Table 1). All samples, including two frozen products, were transported refrigerated and stored at 4°C until processing. Sample processing took place within the minimum shelf life range and latest five days after purchase.

2.2. Sample preparation

In total, 10 g of each sample, representing all layers of a product, were placed in sterile Stomacher bags and diluted with 90 ml sterile PBS. A Stomacher … was used to mechanically comminute the samples for 120 s. To remove coarse food particles, 45 ml of the homogenate was centrifuged at 300 relative centrifugal force(rcf) for 2 min at room temperature using an Eppendorf Centrifuge 5810R and an A-4-62 rotor. The remaining supernatants were transferred to new tubes and centrifuged at 3,000 rcf (30 min at RT). The obtained cell pellets were diluted 10-times with sterile phosphate buffered saline (PBS).

2.3. Bacterial and fungal isolation

We carried out two sets of cultivation experiments. The first set was more general with a basic set of non-selective and selective media (Columbia agar, Violet Red Bile Dextrose agar (VRDB), brain heart infusion agar (BHI), plate count agar (PCA), Rose Bengal (RB), and Baird Parker (BP) agar), to get a broad range of the expected microbial community. The plates were inoculated with a 102 dilution and aerobically and semi-anaerobically incubated at 37°C. In order to recover high amounts of different isolates, the plates where incubated for 16-68 hours, depending on colony size and growth density. Samples with too high growth densities after 16 h for picking single colonies were diluted 103 or 104 in sterile PBS and plated again. For the second media set we used the information from the 16s rRNA ampicon sequencing

to select specific media and growth conditions in order to isolate representatives of genera we could not isolate under the first set's conditions. Depending on the sample specific microbial composition we used Luria Bertani-, nutrient-, tryptic soy-, marine-, corynebacteria-, De-Man-Rogosa-Sharpe- and pseudomonas agar and cultivated the samples (dilution 102-105) anaerobically at 25°C for 48h. In both sets we selected morphologically unique, single colonies for re-cultivation and Sanger sequencing. DNA was extracted, using a protocol modified after Walsh et al. (Walsh et al., 2013), by lysing pure cultures with 100μl 0.01M Tris-HCl and 400μl 2.5% Chelex 100 resin solution (BioRad) at 95°C for 10min, followed by centrifugation with 15,000 rcf for 30 sec. The supernatants were subsequently used for 16S rRNA gene PCR, using a final concentration of 200nM of each of the universal Primers from LGC (27F – 5'-GAG TTT GAT CMT GGC TCA G-3' and 1492R – 5'-GGY TAC CTT GTT ACG ACT T-3'), 0.025 U/μl Invitrogen^{TM} Platinum^{TM} Taq DNA-Polymerase, 1x TaqMan PCR buffer, 2mM MgCl2, and 250nM dNTP Mix (Thermo Scientific^{TM}). For the reaction a protocol of 95°C for 5 min (Taq activation) followed by 35 cycles of 40 seconds at 95°C (denaturation) 40 seconds at 52°C (annealing) and 1 minute at 72°C (elongation) was used. For negative control a reaction with ddH20 as well as a reaction the negative extraction control were performed. In-house Listeria monocytogenes DNA served as positive control. All PCR products were checked with a QIAxcel DNA High Resolution Kit in a QIAxcel Advanced system. Negative PCR results were followed by an ITS2 gene PCR (200nM of each of the primers ITS3-5'-GCATCGATGAAGAACGCAGC-3' and ITS4-5'-TCCTCCGCTTATTGATATGC-3' (White et al. 1990), 0.025 U/μl Invitrogen^{TM} Platinum^{TM} Taq DNA-Polymerase, 1x TaqMan PCR buffer, 2mM MgCl2, and 250nM dNTP Mix (Thermo Scientific^{TM}), with a protocol of 95°C for 5 min (Taq activation) followed by 30 cycles of 40 seconds at 94°C, 40 seconds at 56°C and 1 minute at 72°C). For cultures, negative in 16S rRNA and ITS2 PCR, we repeated the extraction with the NucleoSpin tissue kit (Machery-Nagel), using the manual in combination with the recommendations for hard to lyse bacteria. LGC Genomics GmBH (Berlin) purified and sequenced the PCR products in one direction (using 27F primer for 16S rRNA and ITS4 for the ITS2 region). For quality trimming we used "method 2" (with a defined sliding window of 15 bp and an Phred score mean quality of 40) within the "SangerRead" function ("sangeranalyseR" package; R [**?** Core Team 2021)). Trimmed sequences, with >100 bp length, were classified with the "assignTaxonomy" function of the "dada2" package in R (with kmer size 8 and 50 bootstrap replicates), based on a RDP Naïve Bayesian Classifier algorithm (Wang et al. 2007). Potential pathogens and unclassified Enterobactericeae were further whole genome sequenced on a MinIon MK…

2.4. Bacterial quantification and composition

For direct DNA extraction the DNeasy PowerFood kit was used. That for, the in RNAlater stored cell pellets were thawed, centrifuged (3,000×g, 30min) and resuspended in ??? μl MBL buffer. Deviating from the DNeasy PowerFood protocol, the lysis step was proceeded in … tubes. The further steps were processed according to the protocol. The elution step was done twice with 30μl sterile water, respectively. Parallel an extraction of pure water was performed as negative extraction control (NEC) Sequencing libraries of the 16S rNRA gene (V3/4 region) were prepared based on Illumina 16S Metagenomic Sequencing Library Preparation recommendations. Primers 341F (5 -CCTACGGGNGGCWGCAG-3) and 805R (5 -GAC TAC HVG GGT ATC TAA TCC-3) (Klindworth et al. 2013) were used together with Illumina adapter sequences (5 CGT CGG CAG CGT CAG ATG TGT ATA AGA GAC AG-3 and 5 GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G-3 , respectively) for amplification. Libraries were constructed by ligating sequencing adapters and indices onto purified PCR products using the Nextera XT Sample Preparation Kit (Illumina). Equimolar amounts of each of the purified amplicons were pooled and sequenced on an Illumina MiSeq Sequencer with a 300-bp paired-end read protocol. 16S rRNA gene amplicon library generation and sequencing was performed at the Vienna Biocenter Core Facilities NGS Unit (www.vbcf.ac.at).

2.5. Sequence Processing and Statistics

The data obtained from the MiSeq sequencing went through a Qiime2 workflow including demultiplexing and DADA2 denoising and QC filtering. The taxonomic classification was done with the Scikit-learn algorithm using a pre-trained full-length-uniform-classifier based on the SILVA 138.1 database. After removal of sequences with mitochondrial or chloroplastic origin, further analyses were performed with R. To estimate the alpha-diversities of the samples Hill-Simpson and Hill-Shannon diversity were calculated using the "iNEXT" package, based on 99.5% coverage rarefied samples (Roswell et al. 2021). Group comparisons were done with Kruskal-Wallis tests followed by Bonferroni-alpha-corrected Dunn's tests for pairwise comparisons. For beta diversity analyses the samples were rarefied with 100 iterations based on a coverage of 99.5% using the package "phyloseq" and "metagMisc" [**?** und Jost 2012). Based on that we generated distance matrices with Bray-Curtis dissimilarities, Jaccard indices and Jensen-Shannon divergence and use them for graphical (t-distributed stochastic neighbor embedding - tSNE) and statistical (PERMANOVA, LEfse) analysis. For tSNE we used the "Rtsne" package with a maximum of 1000 iterations, a perplexity of 5 and two initial dimensions, as recommended by Oskolkov [**?** 2019). The high variability of the products and little knowledge on the underlying production conditions made it impossible to find adequate variables for

PERMANOVA, but the main protein source and the texture. The producing facility as additional variable would be meaningful, but the product assortment is dominated by one company, which made the model design very unbalanced. PERMANOVA was done with the "vegan" package using "betadisp" to check for homogeneous dispersion and "adonis" functions for PERMANOVA with 999 iterations using main protein source and texture as explanation variables. Lefse was done with the relative abundance data of the coverage rarefied data in combination with "phyloseqCompanion" R package, for data transformation and the Lefse Bioconda tool by …, using the protein source and texture as class, a normalization of 1 million and a $\log_{10}$ LDA score threshold of 4.0. In parallel, a group comparison for the same features, as examined with Lefse, was done with Kruskal-Wallis tests followed by a Benjamin-Hochberg alpha correction. The Sanger sequences from the Isolates were trimmed using the "sangeranalyseR" package with a Quality Score Cutoff of 40 and a sliding window size of 15. Sequences with a minimum length of 100bp and a trimmed mean quality score >35 were used for taxonomic classification based on the SILVA 138.1 database. Further, the isolate sequences were assigned to a database generated from the MiSeq data to connect the culture-based and culture-independent approaches. For figure 1, the isolate sequences of each genus were clustered within each sample, group and producer using the IdClusters function from the "DECIPHER" package with a cutoff of 0.06. More clusters within each genus were interpreted as a higher species or strain diversity within each genus. Selected isolates were whole genome sequenced with the MinIon, using… The obtained data were trimmed and filtered with filtlong, assembled with Flye followed by several polishing steps (four repetitions of racon and a final step with medaka), before they were used in the TORMES workflow.

3. Results

3.1. Product descriptions

The four groups did not have the same sizes, since the product portfolio for each group was not large enough. Still, we had an overall of 16 pea-based and 16 soybean-based products (Table 1). Beside the four main properties, the products were quite diverse. Particularly noteworthy is the number of ingredients used per product (4-27, with a total of about 120 different used ingredients within the 32 sampled products) and the large range of shelf life. The products are also different on factors, which might influence the bacterial composition, i.e. pre-heating or freezing steps, as well as packing in modified atmosphere. Most of the products had clear cooking instructions on the labels, including the recommendation for thorough cooking (Table 1). In total, 27 samples were packed in modified atmosphere with unknown composition. Out of 30

samples sold refrigerated (the other two frozen) six products were frozen at any point during the retail chain (Table 1).

3.2. Cultivable microbial communities

In total, 465 colonies were picked and selected for 16S rRNA or ITS gene sequencing. Among these, 431 could be classified to the genus level, representing 38 genera in four different phyla (Figure 1). The remaining isolates could only be assigned to family level (n=16, all Enterobactericeae), were fungi (n=15; Wickerhamomyces (n=7), Issatchenkia (n=6), Yarrowia (n=1), Dipodascus (n=1)), or stayed unassigned (n=3). We isolated Bacillus from 19, Leuconostoc from 18, Enterococcus from 12, Latilactobacillus from 10 samples. Species from the genus *Bacillus*, Leuconostoc and Latilacto*Bacillus* could be isolated from each of the four sample groups. *Enterobacteriaceae*, which are usually surveyed as an additional hygiene criterion, were only found in the pea protein products of a single manufacturer (Figure 1). A selection of these *Enterobacteriaceae* and isolates classified as potential pathogens (i.e. *Staphylococcus aureus*, *Bacillus cereus* group, *Klebsiella* sp.) were whole genome sequenced with a MinION device. DATA MISSING

3.3. Lactic acid bacteria and gamma-Proteobacteria *dominate the 16S rRNA amplicon sequences*

In total, 28 samples (883,866 sequences; median frequency per sample: 25,627; range: 439-253,681) passed the quality criteria and were processed with QIIME 2. Because we used coverage based rarefaction (with a coverage of 99.5) and all of the remaining samples meat this coverage, we removed none of them for further analysis. Over all samples, the ASVs were assigned to 25 different Phyla, however, only three Phyla with >3% in at least one of the samples were found (i.e. *Firmicutes* 0.00-0.95%, *Proteobacteria* 0.00-0.31%, *Bacteroidota* 0.00-0.11%). In total, 18 samples were dominated (>50% relative abundance) by *Firmicutes* (10 samples with >90%), while in the other 10 samples *Proteobacteria* is the most abundant Phylum. The most common genera were *Leuconostoc* (detected in 26 samples; 0.03-100.00% rel. abundance), *Latilactobacillus* (detected in 21 samples; 0.02-86.38% rel. abundance), *Pseudomonas* (detected in 21 samples; 0.36-35.25% rel. abundance), *Serratia* (detected in 19 samples; 0.03-8.92% rel. abundance), and Acinetobacter (detected in 18 samples; 0.09-15.40% rel. abundance). The genus Leuconostoc was the most abundant in 13 samples, followed by Latilactobacillus (4 products), and Shewanella (4 products) (Figure 2). Some genera were found proportionally high (10%) in one or more samples, but could not be isolated (i.e. *Shewanella*, *Xanthomonas*, *Photobacterium*, *Myroides*, *Pediococcus*).

3.4. Protein source and texture are not the main driver for the community pattern

The Kruskal-Wallis tests comparing the alpha-diversity indices (i.e. Hill-Shannon index and Hill-Simpson index) between the four groups (based on proteins source and texture) were significant (p.value = 0.018 and 0.049), but in post-hoc Dunn's test with Bonferroni alpha adjustment only the Hill-Shannon index between groups "pea-fibrous" and "pea-minced" differed significantly (p.value= 0.015 - Supplement Figure xy). The group dispersions were homogenous in all examined distance methods (i.e. Bray-Curtis, Jaccard, JSD). The PERMANOVA showed that texture and protein source significantly affects the microbial composition (Supplement Table 2), but explained only between 15.4629221and 22.7898534% of the total variance. The variance explanation by the PERMANOVA would increase, if the manufacturer as variable was added to the model, but since the sampling was very unbalanced on that, we avoided this step.

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3.5. 16S rRNA sequencing revealed three distinct community profiles

In the tSNE plot we see three distinct clusters (Figure 3), which we described, based on the dominating genera as *Leuconostocaceae*-, *Latilactobacillus*- and *Proteobacteria*-profiles. The clustering is traceable, when comparing the similarity of the relative abundance patterns of the samples within each cluster. underlined the heterogenic microbial pattern of Figure 2. Although there is some clustering, there is no clear separation based on the examined variables (main protein source, status, manufacturer).

ALPHA DIV AND LEFSE FOR PROFILES LEfSe identified 32 discriminative features with an LDA Effect Size >4.0 (Fig 3). This analysis highlighted a predominance of Leuconostocaceae (log LDA 5.48) and its classification levels above in pre-cooked pea products. It identified *Proteobacteria* (log LDA 5.33) as most characteristic for raw pea products, mainly based on the predominance of Pseudomonas (log LDA 4.81). Highly discriminant for soy products were some low abundant features. LEfSe calculated and log LDA score of 4.68 for Acetobacter in pre-cooked soy products and of 4.62 for Flavobacteriaceae in raw soy products.

4. Discussion

Highly processed food, like the plant-based meat alternatives we examined in this study, brings challenges to microbiologists. The broad range of ingredients (~120 in in the 32 examined products),different processing steps, and a variety of equipment lead to many potential sources for bacterial contamination. The product specific production processes were not available, but up to the present low and high moisture extrusion are most common commercially used technologies to produce meat textured plant proteins. Both

methods are based on an interaction of heat, shear forces and pressure. The conditions for this process depends on the original protein source and the desired protein structure. During the extrusion, the protein dough undergoes temperature gradients between starting at room temperature and ending between 130 and 160°C at a pressure of…. Blabla et al., described, that the dough remains about two minutes at the final high temperatures. Currently, we assume, that only spore forming bacteria like *Bacillus* would survive the extrusion process. That is also described by Domig et al., who could only isolate *Bacillus* spp. from extruded proteins…(i.e. *Bacillus* and *Clostridium*) can survive (Leutgeb, 2017). This could be one explanation why was isolated from most of the samples (19/32), while the relative abundance of 16S rRNA gene DNA is relatively low, compared to other genera. Overall, we assume, due to this initial extrusion procedure, the main protein is not the source for most of the living bacterial cells we have isolated from the products. The extrusion process (and other heating and freezing steps during the production) brought challenges for the interpretation of sequencing data. It is in the principle of the method that it cannot differentiate between living and dead, which raises the question of whether the use of 16S rRNA metagenomics is an appropriate means of investigating highly processed foods. Along with these processes, there are many changes in the environmental conditions for the products and their associated microbial communities, like heating, thawing, freezing and cooling steps, but also packing in modified atmosphere. These conditional changes could kill or inactivate bacterial cells, while their DNA remains largely intact, but could also induce viable but nonculturable states (Li et al., 2014; Zhao et al., 2017). We agree, that it should not be used as standalone method, but it i) supports culture-based methods and allows to adapt media and culture conditions to genera we find in the sequencing data, which is especially important for less examined environments (i.e. new foods), ii) is able to detect viable but non culturable bacteria, and iii) uncovers contaminations during the process, which could help to adapt HACCP concepts. In this study we examined nine samples with high relative abundancies of *Proteobacteria*, mainly *Pseudomonas*, *Psychrobacter* and *Shewanella*. These three genera, are common spoilers in meat and fish products (Odeyemi et al., 2018)., especially in products with slightly higher pH values, like fish, sea food and poultry. Plant-based meat products are usually also slightly higher in pH (Quellen). Although the number of ASVs within a these genera is much higher, compared e.g. with *Leuconostoc* from other samples, what we associate with different sources of contamination, there are still single ASVs with relatively high abundancies, which indicates either a large entry from one source or an active growth on the product at some time point during the production. The most obvious sources would be the main protein, the used water (via biofilms in water reservoirs or water hoses) or biofilms during the process. From the found genera in the MiSeq data, at least 13 are described as biofilm builders in food

processing environments (Wagner et al.), among these *Pseudomonas*, *Psychrobacter* and *Shewanella*. The possibility of biofilm formation on the processing equipment is high, since many of the used machines are hard to clean. However, this is contradicted by the fact that among the nine *Proteobacteria*-dominated samples seven had a very similar pattern, but were from six different producers. There are no available studies on the microbial communities of raw soybean or pea proteins, but 16S rRNA patterns of peas or the phyllosphere of soy do not support the thesis, that this kind of contamination is associated with the main protein source. Only sample B4, which is the only sample, that is dominated by *Alphaproteobacteria*, had high similarities to the microbial community of the soy phyllosphere (Vorholt, 2012). However, some of the producer just have a few products in this food segment, so we would not exclude, that they buy already extruded proteins from a large distributor and use it for their products.

The comparison of the diversity of the different groups is limited. Since the study design was focused on describing the microbiota of different meat alternatives and to detect possible differences between main protein sources and texture, it was not quite balanced in other perspective. The two most frequently represented manufacturers contributed 18 (11+7) out of 32 products in this study, while the other seven manufacturers contributed with a maximum of two samples. As shown in Supplements Table 1 manufacturers have usually specialized their products to one of the four examined groups. We assume that the production plant has a non-negligible effect on the product's microbiota, which could be seen in part in Figure 3. Based on the microbial distribution patterns from the MiSeq data, we roughly describe three different community compositions. The *Leuconostocaceae* dominated samples (14/27), the *Latilactobacillus* dominated samples (4/27) and the *Proteobacteria* dominated samples. In the first two groups each sample is clearly dominated by one ASV respectively. That speaks for the fact, that there was an active and growing population at any timepoint on the product. Beside of three samples, the dominating ASV assignable to an isolate, therefor we assume, that these species are actually active in the final product. This is especially true for the Lactic acid bacteria. The role of the isolated and sequenced LABs (especially *Leuconostoc mesenteroides* and *Latilactobacillus sakei*) on this product group has to be assessed from several perspectives. They are fermenters on many food products and contribute there to the formation of substances relevant to taste. They inhibit with their metabolites (i.e. organic acids) and their secondary metabolites (i.e. Bacteriocin) the growth of pathogens and spoilage bacteria, but they are spoilage bacteria themselves, especially on meat and meat products. Most of the isolated LABs have ambiguous positions in foods and food production. While *Latilactobacillus* and *Leuconostoc* were used for fermentation of tofu, tempeh or Kimchi, they were also commonly described as food spoilers in non-fermented products, like fresh meat (Casaburi et al., 2015).

Since the examined products were neither assignable as fermented food, nor had fermented products as ingredients (besides vinegar), we associated the isolated genera primarily in their position as product spoilers and not as part of a planned fermentation process. In 18 out of 27 samples with utilizable MiSeq data were lactic acid bacteria dominating (Leuconostoc 13, Lacto*Bacillus* 4, Weissella 1), which is expectable as LABs are described as dominating species on fruits and vegetables [**?** et al. 2020). While ten of the Leuconostoc dominated products were pea protein based, all four *Latilactobacillus*-dominated samples were soy products. The microbial community of all of the pea products with fibrous structure had >95% abundances of LABs (*Leuconostoc*, *Latilactobacillus*, *Weissella*, *Carnobacterium*). *Leuconostoc* is described to have a starting advantage over other LABs on plant products, based on its ability to … According to that Leuconostoc is often described as dominating LAB at the beginning of a fermentation process.

Diverse *Latilactobacillus sakei* strains wurden erfolgreich auf verschiedensten Produkten als Biokonservativ getestet und ist im Regelfall im Vorteil gegenueber Leuconostoc. Zum einen spielen beide Spezies eine Rolle in der Fermentation von diversen pflanzlichen Produkten. In Kimchi ist Leuconostoc in den ersten Tagen, der dominierende LAB und wird zunehmend von *Latilactobacillus sakei* verdraengt [**?** et al. 2013) Gaaerts et al., 2020 examined ready to vegetarian, vegan and insect based meat alternative, including products with fermented ingredients, like fermented sour dough. Based on that, they assumed sour dough as main source for *Latilactobacillus sakei*, which they isolated most. Different to that study, we excluded products with fermented ingredients, but vinegar. Still we found large amounts of lactic acid bacteria in the examined products. Unlike Graeerts et al., we isolated Leuconostoc mesenteroides from the majority of the samples. This species is an obligate heterofermentative LAB, using the phosphoketolase pathway for glycolysis, with lactic acid, acetic acid, CO2 and ethanol as metabolites[**?** 2015). … While *Bacillus* spp. were frequently described as spoilers in bakery and dairy products, but also in meat and poultry, Leuconostoc spp. were mainly associated with the spoilage of meat and seafood but also of vegetables (minimally processed and ready to eat products (Lianou et al., 2016). Lacto*Bacillus* (meat, seafood) pseudomonas (meat, seafood, milk). Undoubtedly, some manufacturers are guided by those similarities when determining their products shelf life and consumption recommendations. Two manufacturer labelled all their products with expiry dates, the others with best before dates. We think that because of the novelty of these products and accompanying missing experience, consumers might not be able to recognize spoiled products by odor or taste. However, broader investigations tailored to this product category and considering the manifold microbial sources, could increase product stability, facilitate manufacturer's decision for product labelling, and help consumer to recognize spoiled products. Altogether, could reduce food waste and increase sustainability of

these products. Beside the dominating, spoilage associated genera, we alos isolated some Enterobactericeae (i.e. Raoultella, Kosakonia, Klebsiella, Escherichia/Shigella, Enterobacter, Citrobacter, Atlantibacter). All of these isolates came from products of the same manufacturer. In MiSeq the relative abundances of *Enterobacteriaceae* were very low in all examined samples. All products were labelled with cooking instructions, most of them specific with cooking time in minutes, others only with "heat through before consumption". This final heating step by the consumer can be a part of the HACCP concept of the manufacturer. While cooking time in minutes is a good guidance for the consumers, "heat through" alone is in our opinion too less information. First there is a lack of experience with these kind of products, second in contrast to real meat (products) there is no indicator like color change for most of these products to determine a sufficiently cooked state. *Enterobacteriaceae* widely serve as hygiene indicators. According to the sequence data *Enterobacteriaceae* had a minor role in the examined samples. However, we isolated seven different genera of Enterobactericeae, among these Enterobacter, Salmonella, Klebsiella and Citrobacter. This indicates, that the conditions in this product group are given for the survival of *Enterobacteriaceae*, including potential human pathogenic species like Enterobacter aerogenes, Salmonella enterica, Klebsiella oxytoca and Citrobacter freundii. Knowing this, we would recommend process hygiene criteria for this product group and offering preparation instruction (incl. cooking time) for all of the products.

General statements about the microbiological risk of these products are difficult to make, since producers, consumers, regulatory authorities, and scientist are not quite experienced with these kind of products. (gueltige regulierungen, haccp concept) In terms of product safety, four critical issues are in the hands of consumers: spoilage detection, handling of the products and used utensils before cooking, the cooking/heating step and the storage for later consumption. Spoilage detection: 2 producer with expiration dates (orientierung an faschierten) rest laesst es den Konsumenten. Keine Erfahrungswerte unmoeglich am geruch zu erkennen, jedes produkt andere rezeptur. Mhd produkte tendetiell hoehere BCE. Große schwankungen in den shelf lifes. Handling of the products: kaum Erfahrung. Wird es wie Fleisch behandelt oder eher nachlaessig. Allgemeine Kuechenhygiene, eigene utensilien bzw. wschschritte handhygiene etc. wir sehen das pathogen in den rohen produkten wachsen koennen. Selbst wenn fleisch erhitzt wird bei fehlender kuechenhygiene hab ich das dann halt wo anders Cooking/heating step. Most oft he products have cooking instructions. Studie hat gezeigt dass die standard pathogene absterben wenn man die produkte wie fleisch entsprechend erhitzt. Fehlende instructions nicht befriedigend. Nur durcherhitzt verzehren als instruction zu wenig, da aqnders als bei Fleisch eine durcherhiztung optisch nicht erkannt werden kann Storage for later consumption: studie der ungarn. Halten tendentiell kuerzer als die fleisch pendants im kuehlschrank nach der zubereitung.

5. Conclusion

6. Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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8. Contributions of Authors

9. Tables

10. Figures

11. Supplements

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