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Microbial Spoilage of Plant-Based Meat Analogues

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Abstract: Plant-based meat analogues (i.e., plant-based meat alternatives or substitutes, or vegan meats) are becoming more and more popular. The quality of the available products is constantly increasing therefore their consumption is also increasing. The primary role of meat analogues is to replace the meat component in meals while appropriate nutrient content and hedonic value will be provided as well. The food safety aspects of these newly emerging food products are less investigated. The aim of this study is to compare the microbial spoilage of identical meals prepared with meat and meat analogues to evaluate the food safety risk of meat analogues. In this work, raw protein materials were tested. Moreover, three pairs of meals prepared with or without meat were microbiologically examined during a storage experiment. Microbial contaminants were low in raw protein sources. In the case of hot meals, the microbial proliferation was faster in samples containing meat analogue, especially if the meals were not cooled. The food safety risk of meals prepared with meat analogues is slightly higher than their meat-containing counterparts, therefore more attention needs to be paid to the preparation, processing, and storage of these foods.

Keywords: meat analogue; microbiological spoilage; aerobic colony count; food safety risk; meat substitute; meat alternative; vegan meat

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1. Introduction

Eating habits have considerable impact on the climate and on the efficient utilization of natural resources. The food sector is one of the major environmental burdens, where the majority of the products is of animal origin. Meat has been a significant food of mankind for a long time, and it is an important component of the diverse and well-balanced diet. Meat has an inevitable role in protein intake. The food consumption level of a country is often related to the social development [1], so it can be concluded that social development is usually manifested in elevated meat consumption [2]. Meat consumption in Europe is 69 kg/per capita/year on average [3].

The rapid increase in meat consumption shed light on the resource demand of meat production. Cattle are responsible for about 6.52% of total anthropogenic greenhouse gas emissions (3.19 Gigatons of carbon dioxide equivalents) [4,5] and the production of 1 kg of beef causes 26.5 kg CO_2 emission and needs 15,000 litres of water (equal to 50 days' household water use per capita in EU) [6,7]. Many researchers suggest that meat consumption need to be decreased to protect and preserve the environmental resources.

Decreasing global meat consumption would cause a significant positive effect on the environment [8–10].

Meat consumption also contributes to public health issues. Excessive meat consumption may bring serious harmful health effects according to scientific literature. Red meat consumption was shown to be a major cause of cardiovascular diseases (hypertension, atherosclerosis, etc), chronic inflammatory conditions, and Type 2 diabetes [11–13]. Eating less meat significantly decreases the risk of cardiovascular conditions, obesity, diabetes, and cancer [14].

Plant-based nutrition is popular worldwide: 45% of the younger generation regularly eat vegetarian and vegan food, and a full quarter of 25–34-year-old Americans are vegans or vegetarians [15]. Although the number of vegans and vegetarians are rapidly increasing, the plant-based market is driven by followers of the flexitarian diet. Meat reduction is proving to be a bigger trend than meat avoidance [16].

However, many people are reluctant to change their diet to eat less meat [17–19]. A survey conducted in Germany and New Zealand revealed that 14–18% of consumers are willing to try innovative food and replace meat; however, 25–30% of consumers do not think about meat replacement with meat analogue products [20].

There are many ways to decrease meat consumption level, and a common method is the replacement of meat ingredients with some non-meat component, while keeping the nutritional and hedonic characteristics almost the same. According to the literature, the current meat consumption level could be decreased by the use of plant-based meat analogues, insects, microorganisms, or in the near future, with laboratory grown meat (cultured meat or in vitro meat) [21,22]. Nowadays, the most common means for meat replacement is the use of plant-based meat analogues [23]; thus, we focus on these products in our present research. Meat analogues are different from traditional vegetarian alternatives, promising to be indistinguishable from animal-derived meat. Good-quality meat analogues should reproduce the appearance, texture, smell, flavour, processing behaviour, nutritional values, and enjoyment value of various meat types. Nowadays, several products that meet the above-mentioned criteria are in the market.

The main task in meat replacement is to ensure adequate protein content and to maintain the meat-like taste, texture, colour appearance, and consumer experience. The most frequently used raw materials of meat analogues are cereals and legumes [24,25]. Previously, soybean and wheat gluten were the primary raw materials, but nowadays there are many more alternatives such as maize, rice, barley, oat, sorghum, bean, lentil, pea, lupine, etc. [26]. The protein is extracted from the plant resulting in a protein isolate, protein concentrate, or protein texturate. Plant protein ingredients available for meat analogue production include flours (10–20% protein), concentrates (55–60% protein), isolates (>80% protein), and texturized protein (50–70% protein) [27,28]. The protein ingredients have several key characteristics that primarily determine the consumer's experience. For example, water and oil-holding capacity, solubility, emulsification capacity, foaming property, gelling, etc., influence key characteristics of protein ingredients such as flexibility, formability, shape retention, etc. These attributes are related to amino acid sequence, secondary, or higher structure, but environmental circumstances such as pH, temperature, and ionic strength also can alter the protein structure [26].

Since a direct link was discovered between human health and animal health, meat safety has been of interest, and since then, meat safety systems have been constantly evolving [29]. Meat analogues are becoming more and more similar to real meat, but it is still questionable whether their food safety risks are the same. Although good-quality products are available on the market, their food safety aspects are less investigated. The aim of this work was to evaluate the microbial food safety risk of plant-based meat analogues. For this purpose, the microbial status of raw materials and the microbiological spoilage of hot meals were studied.

2. Materials and Methods

2.1. Sample Material

Two sample types were involved in the experiment. At first, raw materials suitable for meat analogue production were investigated. Secondly, three types of hot meals were prepared with meat and a meat analogue.

2.2. Protein Raw Materials

Five raw protein materials were investigated as follows:

1. Wheat gluten powder.
2. Textured soy protein.
3. Soy protein powder.
4. Textured pea protein (70% protein content).
5. Textured pea protein (55% protein content).

The products were acquired from various manufacturers (Vestkorn, Dupont), all of which were within their shelf-life period.

2.3. Hot Meals

Three kinds of hot meal were prepared, all in two versions, with and without meat, so six samples were involved in our experiments. The meat analogues used were prepared from textured pea protein (55%) according to our own recipe. The hot meals and information on their ingredients and nutritional characteristics are shown in Table 1. The meatless foods are marked with the vegan prefix.

Table 1. Food samples, ingredients, and nutritional data.

	Food	Energy (kJ)	Protein (g)	Fat (g)	Carb (g)
1.	Spaghetti Bolognese (minced beef, salt, tomato puree, onion, carrot, olive oil, spices)	528	11.05	2.55	13.15
2.	Vegan spaghetti Bolognese (textured pea protein 55%, salt, tomato puree, onion, carrot, olive oil, spices)	613	11.57	3.33	21.96
3.	Cabbage casserole (minced pork, cauliflower, rice, egg, onion, sour cream, olive oil, salt, spices)	456	8.60	2.93	10.89
4.	Vegan cabbage casserole (textured pea protein 55%, coconut fat, corn fiber, corn starch, methyl-cellulose, cauliflower, rice, sour cream, olive oil, salt, onion, spices)	471	13.61	1.22	10.07
5.	Meat ball (minced beef and pork, egg, salt, onion, garlic, spices)	817	20.26	6.73	15.97
6.	Vegan meat ball (textured pea protein 55%, coconut oil, corn fiber, corn starch, methyl-cellulose, salt, fat, onion, garlic, spices)	689	10.98	9.57	9.37

The six food samples were prepared simultaneously. The sauce of spaghetti Bolognese was gentle boiled for 75 min, pasta was cooked in boiling water for 12 min, then the pasta and sauce were mixed, and additional ingredients (e.g., parmesan) were not added.

Cabbage casserole was heat treated for 40 min at 170 °C in a combi oven. Meatballs were prepared in a deep fryer at 170 °C for 10 min. All products were heat treated only once.

After preparation, 100 g portions were distributed into sterile containers. Samples were stored at ambient temperature and at 5 °C.

2.4. Storage

Some of the food samples were stored at ambient temperature (20–22 °C). These samples were taken for microbial investigation every 12 h.

The other part of the samples was quickly cooled after preparation and stored at 5 ± 2 °C. Samples were taken for microbial investigation every 24 h.

2.5. Microbial Investigation

Sample preparation: Ten grams of the food sample were mixed with 90 mL peptone water and homogenized in a stomacher. Ten-fold dilution series were then prepared. The diluted samples were then surface plated on agar-plates.

Aerobic colony count: The samples were plated onto plate count agar according to ISO 4833-2:2013 [30]. Briefly, 0.1 mL test samples of each dilution were transferred and spread with a sterile spreader on plate count agar (PCA) plates and incubated at 30 °C for 72 ± 3 h. After the incubation period, colonies were counted and the results were expressed as $\log_{10}$CFU/g.

Yeast and molds: According to ISO 21527-1:2008 [31] and ISO 21527-2:2008 [32] standards, the diluted samples were transferred and spread on DRBC (dichloran rose bengal chloramphenicol agar) or DG18 agar plates (depending on the food matrices) and incubated at 25 °C for five days. The developed colonies were counted, and the results were expressed as $\log_{10}$CFU/g. Yeasts and molds were distinguished according to colony morphology.

Enterobacteriaceae: Following the procedure of ISO 21528-2:2007 [33] standard, 1 mL test samples of each dilutions were transferred to sterile Petri dishes and 12–15 mL violet red bile glucose agar were poured into each Petri dishes. The inoculums were carefully mixed with the medium and allowed to solidify. The plates were incubated at 37 °C for 24 h and the colonies were counted. Typical colonies were confirmed by means of tests for the fermentation of glucose and the presence of oxidase.

All microbiological media were provided by Neogen.

While Enterobacteriaceae are significant in meat hygiene, yeasts and molds are food-safety indicators for plant-based products. However, for comparability, these microorganisms were tested in both product types.

2.6. Statistics

Three independent samples were taken from each food or raw material. The measurements were carried out for each parallel sample. Means and standard deviations were calculated from parallel measurements, and significant differences were evaluated by Student's t-test. Differences were considered to be significant at $p = 0.05$.

3. Results

3.1. Raw Materials

Wheat gluten powder showed a microbial load with the value of 3.32 $\log_{10}$CFU/g for the aerobic colony count, which is considered as a medium-contaminated raw material; however, it indicates elevated food safety risk. In all other raw materials, the aerobic colony count was below 10 CFU/g. Yeast, mold, and *Enterobacteriaceae* were not detected in any of the raw materials (Table 2).

Table 2. Initial microbial load of protein raw materials.

Protein Raw Material	Aerobic Colony Count	Yeasts and Moulds	Enterobacteriaceae
Wheat gluten powder	3.32 ± 0.91 $\log_{10}$CFU/g	<10 CFU/g	<10 CFU/g
Textured soy protein	<10 CFU/g	<10 CFU/g	<10 CFU/g
Soy protein powder	<10 CFU/g	<10 CFU/g	<10 CFU/g
Textured pea protein (70% protein content)	<10 CFU/g	<10 CFU/g	<10 CFU/g
Textured pea protein (55% protein content)	<10 CFU/g	<10 CFU/g	<10 CFU/g

3.2. Storage without Refrigerating

It was observed in meatless samples that the aerobic colony count started to increase after 12 h; however, the spoilage of meat-containing meals started later (Figure 1, Table S1.) In the case of spaghetti Bolognese, the samples after preparation showed no microbial contamination, but 12 h later, microbial proliferation was observed in the meatless meals. Twenty-four hours after preparation and later, the level of aerobic colony count was similar in meaty and meatless dishes. The results were similar in cabbage casserole; however, microbial spoilage started 36 h after preparation in meatless meals. In meatballs, the differences in microbial load were constant during the whole experiment, but the meatless dish showed higher levels of microbial contamination at all sampling times.

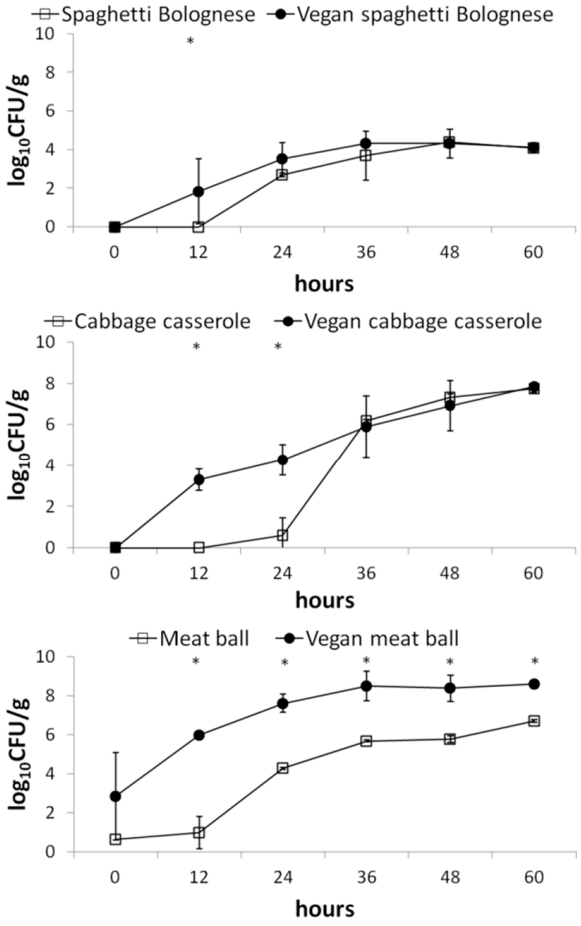

Figure 1. Changes in aerobic colony counts in food samples stored without refrigeration. Mean values ± standard deviations are shown. Asterisks indicate significant differences between meat containing and meatless samples at $p < 0.05$.

Enterobacteriaceae was found only in vegan meatballs in two cases (sample stored for 36 h: $\log_{10}$CFU/g = 5.46; sample stored for 48 h: $\log_{10}$CFU/g = 7.26). Yeast was detected in the vegan cabbage casserole sample stored for 24 h ($\log_{10}$CFU/g = 3.28).

3.3. Storage with Refrigerating

Bacterial counts in the refrigerated samples were less uniform. Microbes were found in all meatless Spaghetti Bolognese samples; however, the contamination level was low ($\log_{10}$CFU/g ranged from 1 to 2). Bacteria were undetectable in meat-containing samples until the fourth day. The cabbage casserole showed low bacterial counts in all samples. Microbial spoilage was detected only in meatball and vegan meat ball samples, but no differences were observed between the two meal types (Figure 2, Table S2.).

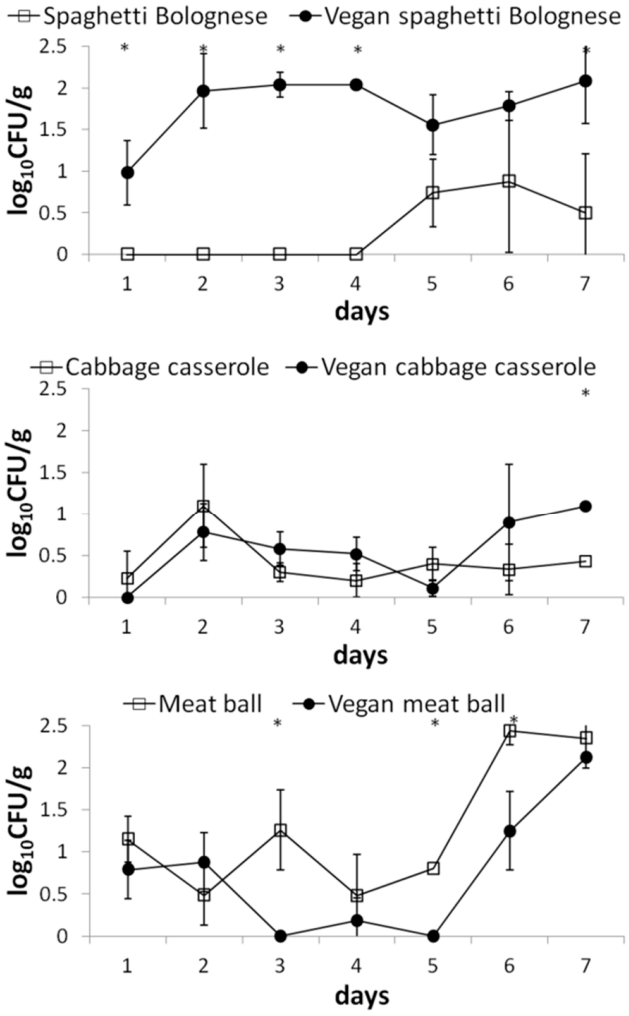

Figure 2. Changes in aerobic colony counts in refrigerated food. Mean values ± standard deviations are shown. Asterisks indicate significant differences between meat-containing and meatless samples at $p < 0.05$.

Enterobacteriaceae were undetectable in all samples, and mold was observed in only one vegan spaghetti Bolognese sample (day 6, $\log_{10}$CFU/g = 3.64)

pH values are shown in Table 3. The pH value did not change during the seven-day refrigerated storage. It can be observed that the pH values of vegan foods are higher and the differences were significant in all cases.

Table 3. The pH values of processed foods.

Food	pH Mean ± StDev *
Spaghetti Bolognese	5.3 ± 0.2
Vegan spaghetti Bolognese	5.5 ± 0.1
Cabbage casserole	5.9 ± 0.1
Vegan cabbage casserole	6.4 ± 0.1
Meat ball	6.2 ± 0.1
Vegan meat ball	6.6 ± 0.1

* Mean ± standard deviation of seven measurements of the seven storage days are shown.

4. Discussion

When we think about meat alternatives, typically plant proteins are considered because they are healthy, cheap, and are already a part of most diets [22].

Nowadays plant-based products regularly enter the market, aiming at replacing meats [34,35]. These products are mainly based on soy, wheat, or pea protein or a mixture of them [36]. The most common meat alternatives are based on soy protein; however, pea protein-based products are gaining more and more popularity [20], because soy and wheat products cause food intolerances for a significant number of customers. Our experiments focused mainly on pea protein-based products.

A consumer survey already revealed that freshness is the second most important food choice criterion, and only the price proved to be more important [37]. However, another important aspect is a long shelf-life. These consumer expectations make great demands on effective management and control of food spoilage. The food microbial spoilage could be caused by enzymatic or microbiological processes but the microbial spoilage affects primarily the product shelf life [38].

Only a few data are available about the food safety risk of plant-based meat analogues. Filho et al. [39] tested the microbial spoilage of soy- and wheat-based canned meat analogues, and the usual heat treatment (121 °C/30 min) proved to be adequate for canned plant-based meat analogues. Yadav et al. [40] compared the microbial spoilage of chicken roll and meatless roll, but no differences were revealed. Due to their high protein and moisture content, and almost neutral pH, the plant-based meat analogues are susceptible to spoilage [41].

The aim of this work was the investigation of the microbiological quality of meals that are directly consumed. If microbial contamination is detected, this is usually caused by raw materials, therefore the evaluation of raw materials' microbial quality is of great importance. Our experiments were set up to observe the microbial spoilage caused only by the intrinsic effects of ready-made meals [42], namely by the ingredients. Therefore, the hot meal pairs were prepared identically, the only difference was in the meat/meat analogue ingredient.

Typical food safety problem of households is the inappropriate storage of cooked food. Samples stored at room temperature and at 5 ± 2 °C could be considered as a simulation of common household situations [43].

The pH values of the vegan meatball and vegan cabbage casserole were higher (0.5 more on the average) than their meat-containing counterparts. The pH values of spaghetti Bolognese were similar, probably due to the significant amount of tomato sauce.

Enterobacteriaceae could be present in food as part of natural microflora, but post-process contamination is the more significant factor regarding food quality. Members of *Enterobacteriaceae* family are responsible for spoilage of variety of foods such as meats, dairies, fruits, and vegetables, etc. They can be found also in soil or water so their control is significant for food hygiene [44]. If *Enterobacteriaceae* are present in heat-treated food, a post-process contamination is implicated. No *Enterobacteriaceae* were detected in refrigerated samples. Two unrefrigerated samples contained *Enterobacteriaceae*, and both samples were

the vegan meatball. It can be assumed that post-process contamination occurred, and ambient temperature is adequate for *Enterobacteriaceae* proliferation [44].

Th presence of molds and yeasts in food is usually caused by raw materials or post-process contamination, therefore mould and yeast counts can be used as a hygiene process indicator primarily in the case of plant-based products. Yeasts were observed in cooled vegan spaghetti Bolognese, probably caused by post-process contamination. Yeasts were also observed in one sample of unrefrigerated vegan cabbage casserole.

The investigated protein raw materials do not pose microbial risk. Microbes were detected only in wheat gluten powder, but microbial loads were below the threshold ($\log_{10}$CFU/g = 5) [45]. Moreover, the raw materials were exposed to heat treatment so they can be considered as safe in microbial aspects.

The obtained results show that in some cases, microbial spoilage is faster in vegan food. The pH of these food is closer to 7 (Table 2), and the high water content and water activity of protein raw material promote bacterial proliferation [38,46].

Yeast and *Enterobacteriaceae* were observed only in meatless foods, suggesting that food safety risk of food prepared with meat analogues is slightly higher than that of meat-containing food. Enumeration of yeasts, molds, and *Enterobacteriaceae* could be used as process hygiene indicators in the production of meat analogues.

5. Conclusions

The current trends in meat production and consumption are environmentally unsustainable, therefore solutions are needed to reverse the trend. One solution could be increasing the consumption of alternative protein sources such as insects, cultured meat, or plant-based proteins. The latter case is boosted by plant-based meat analogue products. Consumer demand for these products is rapidly increasing, but for food processors, it is still difficult to produce good-quality products in the appropriate quantities. Therefore, products must be further developed where the food safety aspects must be considered. Our results show that protein raw materials appropriate for meat analogue production do not pose microbial food safety risk; however, the meals where meat analogues were used proved to be more perishable. An effective food safety management system should be operated to support meat analogue production.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11188309/s1, Table S1: Changes in aerobic colony counts in food samples stored without refrigeration; Table S2: Changes in aerobic colony counts in refrigerated food.

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References

1. Van der Weele, C.; Feindt, P.; Jan van der Goot, A.; van Mierlo, B.; van Boekel, M. Meat alternatives: An integrative comparison. *Trends Food Sci. Technol.* **2019**, *88*, 505–512. [CrossRef]
2. Keefe, L.M. #FakeMeat: How big a deal will animal meat analogs ultimately be? *Anim. Front.* **2018**, *8*, 30–37. [CrossRef] [PubMed]
3. OECD/FAO. *OECD-FAO Agricultural Outlook 2019-2028*; OECD Publishing: Paris, France, 2019.
4. Gerber, P.J.; Steinfeld, H.; Henderson, B.; Mottet, A.; Opio, C.; Dijkman, J.; Falcucci, A.; Tempio, G. *Tackling Climate Change through Livestock—A Global Assessment of Emissions and Mitigation Opportunities*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2013.

5. Grossi, G.; Goglio, P.; Vitali, A.; Williams, A.G. Livestock and climate change: Impact of livestock on climate and mitigation strategies. *Anim. Front.* **2019**, *9*, 69–76. [CrossRef]
6. Mekonnen, M.M.; Hoekstra, A.Y. A global assessment of the water footprint of farm animal products. *Ecosystems* **2012**, *15*, 401–415. [CrossRef]
7. Ogino, A.; Orito, H.; Shimada, K.; Hirooka, H. Evaluating environmental impacts of the Japanese beef cow-calf system by the life cycle assessment method. *Anim. Sci. J.* **2007**, *78*, 424–432. [CrossRef]
8. Willett, W.; Rockström, J.; Loken, B.; Springmann, M.; Lang, T.; Vermeulen, S.; Garnett, T.; Tilman, D.; DeClerck, F.; Wood, A.; et al. Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet* **2019**, *393*, 447–492. [CrossRef]
9. Alexander, P.; Brown, C.; Arneth, A.; Finnigan, J.; Rounsevell, M.D.A. Human appropriation of land for food: The role of diet. *Glob. Environ. Chang.* **2016**, *41*, 88–98. [CrossRef]
10. Springmann, M.; Godfray, H.C.J.; Rayner, M.; Scarborough, P. Analysis and valuation of the health and climate change cobenefits of dietary change. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 4146–4151. [CrossRef]
11. Walker, P.; Rhubart-Berg, P.; McKenzie, S.; Kelling, K.; Lawrence, R.S. Public health implications of meat production and consumption. *Public Health Nutr.* **2005**, *8*, 348–356. [CrossRef] [PubMed]
12. Huang, Y.; Cao, D.; Chen, Z.; Chen, B.; Li, J.; Guo, J.; Dong, Q.; Liu, L.; Wei, Q. Red and processed meat consumption and cancer outcomes: Umbrella review. *Food Chem.* **2021**, *356*, 129697. [CrossRef]
13. Micha, R.; Wallace, S.K.; Mozaffarian, D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus. *Circulation* **2010**, *121*, 2271–2283. [CrossRef] [PubMed]
14. Froggatt, A.; Wellesley, L. *Meat Analogues: Considerations for the EU*; The Royal Institute of International Affairs, Chatham House: London, UK, 2019.
15. Kamila, A.Y. Millennials lead food industry toward greener territory. *Portl. Press Her.* **2015**. Available online: https://www.pressherald.com/2015/09/09/vegetarian-kitchen-millennials-lead-food-industry-toward-greener-territory (accessed on 16 August 2021).
16. ProVeg International. *European Consumer Survey on Plant-Based Foods—Describing the Product Landscape and Uncovering Priorities for Product Development and Improvement*; ProVeg International: Berlin, Germany, 2020. Available online: https://proveg.com/what-we-do/corporate-engagement/proveg-consumer-survey-report-download/ (accessed on 16 August 2021).
17. Macdiarmid, J.I.; Douglas, F.; Campbell, J. Eating like there's no tomorrow: Public awareness of the environmental impact of food and reluctance to eat less meat as part of a sustainable diet. *Appetite* **2016**, *96*, 487–493. [CrossRef]
18. Tuorila, H.; Hartmann, C. Consumer responses to novel and unfamiliar foods. *Curr. Opin. Food Sci.* **2020**, *33*, 1–8. [CrossRef]
19. Onwezen, M.C.; Bouwman, E.P.; Reinders, M.J.; Dagevos, H. A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat. *Appetite* **2021**, *159*, 105058. [CrossRef]
20. Lemken, D.; Spiller, A.; Schulze-Ehlers, B. More room for legume—Consumer acceptance of meat substitution with classic, processed and meat-resembling legume products. *Appetite* **2019**, *143*, 104412. [CrossRef] [PubMed]
21. Alexander, P.; Brown, C.; Arneth, A.; Dias, C.; Finnigan, J.; Moran, D.; Rounsevell, M.D.A. Could consumption of insects, cultured meat or imitation meat reduce global agricultural land use? *Glob. Food Sec.* **2017**, *15*, 22–32. [CrossRef]
22. Fasolin, L.H.; Pereira, R.N.; Pinheiro, A.C.; Martins, J.T.; Andrade, C.C.P.; Ramos, O.L.; Vicente, A.A. Emergent food proteins—Towards sustainability, health and innovation. *Food Res. Int.* **2019**, *125*, 108586. [CrossRef]
23. Gómez-Luciano, C.A.; de Aguiar, L.K.; Vriesekoop, F.; Urbano, B. Consumers' willingness to purchase three alternatives to meat proteins in the United Kingdom, Spain, Brazil and the Dominican Republic. *Food Qual. Prefer.* **2019**, *78*, 103732. [CrossRef]
24. Elzerman, J.E.; Hoek, A.C.; van Boekel, M.A.J.S.; Luning, P.A. Consumer acceptance and appropriateness of meat substitutes in a meal context. *Food Qual. Prefer.* **2011**, *22*, 233–240. [CrossRef]
25. Hoek, A.C.; van Boekel, M.A.J.S.; Voordouw, J.; Luning, P.A. Identification of new food alternatives: How do consumers categorize meat and meat substitutes? *Food Qual. Prefer.* **2011**, *22*, 371–383. [CrossRef]
26. Kyriakopoulou, K.; Dekkers, B.; van der Goot, A.J. *Plant-Based Meat Analogues*; Galanakis, C.M., Ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2019; pp. 103–126. ISBN 9780128148747.
27. Akharume, F.U.; Aluko, R.E.; Adedeji, A.A. Modification of plant proteins for improved functionality: A review. *Compr. Rev. Food Sci. Food Saf.* **2021**, *20*, 198–224. [CrossRef]
28. Cheatham, R. *Protein: A Plant-Based Look at This Power Macronutrient*; Virgo Publishing LLC: Phoenix, AZ, USA, 2013.
29. Blagojevic, B.; Nesbakken, T.; Alvseike, O.; Vågsholm, I.; Antic, D.; Johler, S.; Houf, K.; Meemken, D.; Nastasijevic, I.; Vieira Pinto, M.; et al. Drivers, opportunities, and challenges of the European risk-based meat safety assurance system. *Food Control* **2021**, *124*, 107870. [CrossRef]
30. International Organization for Standardization 4833-2:2013 Microbiology of the Food Chain—Horizontal Method for the Enumeration of Microorganisms—Part 2: Colony Count at 30 °C by the Surface Plating Technique. 2013. Available online: https://www.iso.org/standard/59509.html (accessed on 10 May 2021).
31. International Organization for Standardization 21527-1:2008 Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Part 1: Colony Count Technique in Products with Water Activity Greater than 0,95. 2008. Available online: https://www.iso.org/standard/38275.html (accessed on 10 May 2021).

32. International Organization for Standardization 21527-2:2008 Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Yeasts and Moulds—Part 2: Colony Count Technique in Products with Water Activity Less than or Equal to 0,95. 2008. Available online: https://www.iso.org/standard/38276.html (accessed on 10 May 2021).
33. International Organization for Standardization 21528-2:2017 Microbiology of the Food Chain—Horizontal Method for the Detection and Enumeration of Enterobacteriaceae—Part 2: Colony-Count Technique. 2017. Available online: https://www.iso.org/standard/63504.html (accessed on 10 May 2021).
34. Ingenbleek, P.T.M.; Zhao, Y. The Vegetarian Butcher: On its way to becoming the world's biggest 'meat' producer? *Int. Food Agribus. Manag. Rev.* **2019**, *22*, 295–307. [CrossRef]
35. Kumar, P.; Chatli, M.K.; Mehta, N.; Singh, P.; Malav, O.P.; Verma, A.K. Meat analogues: Health promising sustainable meat substitutes. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 923–932. [CrossRef] [PubMed]
36. Schreuders, F.K.G.; Dekkers, B.L.; Bodnár, I.; Erni, P.; Boom, R.M.; van der Goot, A.J. Comparing structuring potential of pea and soy protein with gluten for meat analogue preparation. *J. Food Eng.* **2019**, *261*, 32–39. [CrossRef]
37. Röhr, A.; Lüddecke, K.; Drusch, S.; Müller, M.J.; Alvensleben, R.V. Food quality and safety—-Consumer perception and public health concern. *Food Control* **2005**, *16*, 649–655. [CrossRef]
38. Nychas, G.-J.E.; Panagou, E. Microbiological spoilage of foods and beverages. In *Food and Beverage Stability and Shelf Life*; Elsevier: Amsterdam, The Netherlands, 2011; pp. 3–28.
39. Filho, G.C.S.; Vessoni Penna, T.C.; Schaffner, D.W. Microbiological quality of vegetable proteins during the preparation of a meat analog. *Ital. J. Food Sci.* **2005**, *17*, 269–283.
40. Yadav, P.; Ahlawat, S.S.; Jairath, G.; Rani, M.; Bishnoi, S. Studies on physico-chemical properties and shelf life of developed chicken meat analogue rolls. *Haryana Vet.* **2015**, *54*, 25–28.
41. Wild, F.; Czerny, M.; Janssen, A.; Kole, A.; Zunabovic, M.; Domig, K. The evolution of a plant-based alternative to meat: From niche markets to widely accepted meat alternatives. *Agro Food Ind. Hi Tech.* **2014**, *25*, 45–49.
42. Lianou, A.; Panagou, E.Z.; Nychas, G.-J.E. Microbiological Spoilage of Foods and Beverages. In *The Stability and Shelf Life of Food*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 3–42.
43. Worsfold, D. Food safety behaviour in the home. *Br. Food J.* **1997**, *99*, 97–104. [CrossRef]
44. Baylis, C.; Uyttendaele, M.; Joosten, H.; Davies, A. The Enterobacteriaceae and their significance to the food industry. *ILSI Eur. Rep. Ser.* **2011**, 9–12.
45. Health Protection Agency of United Kingdom. *Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market*; Health Protection Agency of United Kingdom: London, UK, 2009. Available online: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/363146/Guidelines_for_assessing_the_microbiological_safety_of_ready-to-eat_foods_on_the_market.pdf (accessed on 10 May 2021).
46. Samard, S.; Ryu, G. A comparison of physicochemical characteristics, texture, and structure of meat analogue and meats. *J. Sci. Food Agric.* **2019**, *99*, 2708–2715. [CrossRef] [PubMed]