

Assessing the Microbiological Safety of Plant-Based Alternatives to Meat and Dairy Products

UKHSA annual programme of food and environmental studies

- National studies
 - > All Local Authorities requested to participate
 - > Questionnaires used to gather details relating to samples
 - Pre-planned topics of interest and reactive studies
 - > Aim to publish results wherever possible
- Consultation on short-list of suggestions circulated to stakeholders in November / December each year
- Top two options selected, plus a reactive study dependent on current issues
- Regional studies may be organised by each lab based on local concerns may be useful for local understanding or as pilot studies for future national focus

Protocol shared

- Specifies time period, types of premises
- Sample types included and excluded
- Tests to be performed
- Interpretation of results
- Does not prescribe numbers of samples of each type (i.e. not based on market share etc)
- We accept that our surveys will tend to focus more on higher risk products

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Plant-based diets

- Plant-based diets increasingly popular
- Shown to be healthier
 - significantly reduced risk of negative health outcomes (high blood pressure, heart disease, diabetes)
- Climate considerations
- Increasing availability of meat-free and dairy-free plant-based options

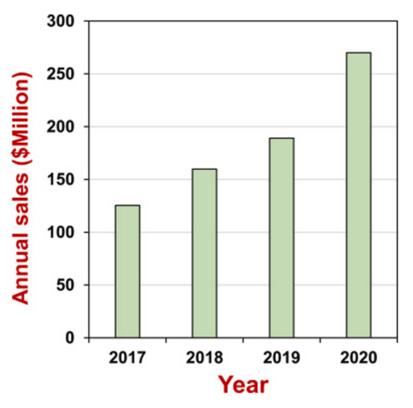


Plant-based milk and cheese sales



By 2019, plant-based milks accounted for 8% of all milk sales in UK

Fig. 1. Annual plant-based cheese sales in the USA



5

How are they made? Plant-based milks

Variations in the manufacturing depending on starting plant material

For soy milk, process involves:

- cleaning, soaking and dehulling the beans
- grinding beans to a slurry
- heating to denature lipoxidase enzymes to reduce effects flavour
- removing solids by filtration
- adding water, sugar and other ingredients to improve flave and micronutrient content; oils / thickening or stabilising agents
- pasteurizing the pre-final liquid

Storage at refrigeration or ambient temperature, depending heating stage

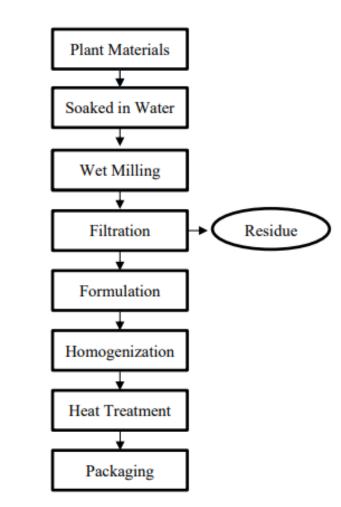


Figure 1. Procedure of plant-based milk production

6

How are they made? Vegan cheeses

- Soaking of nuts (eg cashews or almonds) for at least 4 hours
- Blending (with eg nutritional yeast, lemon juice, vinegar, flavourings)
- Addition of tapioca starch or agar agar powder for more stretchy / melting consistency
- Cook to achieve consistency
- Transfer to mold and let it set

OR

- Fermentation of eg soy milk using starter culture
- Curd cut, pressed and salted



Vegan study

8

(Nicolás Saraco & Blaxland, 2020)

2% sunflower oil-based

6% palm-oil based

10% nut-based

74% coconut-oil based

the market

5% rice-based

3% soy-based

Common plant-based cheese components

Study carried out on commercial plant-based cheeses in UK identified 109 products on



How are they made? Vegan meat substitutes

- Protein source selected (legumes; wheat gluten; soy; algae)
- Texturisation mix protein source with water / other liquid to produce doughlike consistency – subjected to mechanical processing to achieve fibrous texture
- Binders (starches / gums) may be added
- Fats and flavourings added
- Colourings added
- Formed into shapes by extrusion into sausage shape / moulding into burgers etc
- Cooked to develop flavour / texture

9

Potential risks

- Contamination of plant-based ingredients
 - dried pulses / nuts / grains likely to contain spores Bacillus cereus
 - Salmonella previously associated with nuts
 - Yeasts and moulds likely on dried ingredients
- Soaking process may allow growth of bacteria
- Soaking of kidney beans overnight Bacillus shown to grow at ambient temperature but not if soaked in fridge
- Relatively few controls in final products to minimise microbial growth during shelf-life



Salmonella and vegan cheese

2020 / 2021:

- Salmonella outbreak linked to vegan cheese in US
- 20 cases S. Chester, S. Urbana, S. Duisburg and S. Typhimurium!
- Outbreak strains (S. Chester and S. Urbana) found in production environment and in raw cashew nuts used in cheese production
- No pasteurisation step included in processing

2013 / 2014:

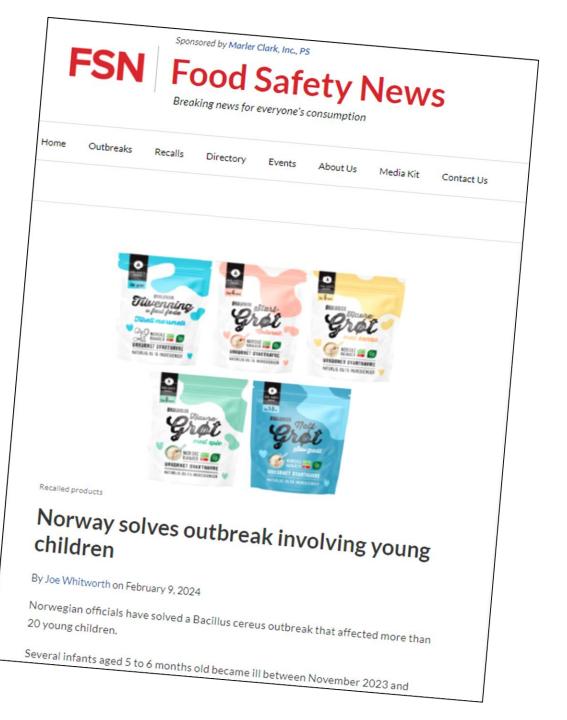
- 17 cases of salmonellosis (S. Stanley) linked to cashew cheese in California
- S. Weltevreden also isolated from fermenting cashew nuts at production premises





Bacillus in oats





Bacillus levels in plant-based ingredients

- Study carried out in Netherlands
- Tested 88 samples of pulses, cereals and drupes (coconut / almond / cashew)
- B. cereus detected at >10 cfu/g in
 - 30% of pulses
 - 13% of cereals (oat ingredients)
- 9% of *B. cereus* strains contained emetic toxin (ces gene)
- 42% /28% / 69% contained different enterotoxins (cytK, hbl, nhe)
- 9% contained both emetic and enterotoxin
- 4% contained no toxin genes

Kyrylenko et al (2023) Int J Food Microbiol

Study 75: Ready to eat plant based meat, fish and dairy substitutes - September 2022 to March 2023

- Meat alternatives *e.g.* salami style, chicken style, vegan sausage rolls, pepperoni style, tofu based, *etc*
- Fish alternatives *e.g.* vegan salmon, vegan shrimp, etc
- Dairy alternatives *e.g.* Plant based cheese, milk, cream, yoghurt *etc*
- From any retail or catering premises
- NOT: products needing further cooking or processing (*e.g.* soya or Quorn mince) or products with multiple ingredients *e.g.* meals.

Test for:

- Salmonella
- Listeria detection and enumeration
- Enterobacteriaceae
- Escherichia coli
- coagulase positive *Staphylococcus*
- Bacillus cereus
- Aerobic colony count (ACC)
- pH
- Water activity (a_w) for all products except milks and other liquids

Samples collected

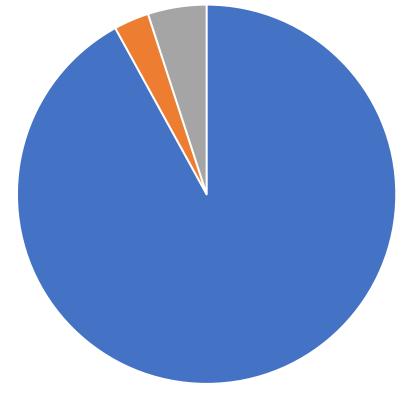
- 937 samples:
 - ≻44% meat substitutes
 - ≥26% vegan cheeses
 - >15% plant-based milks
 - >12% other dairy alternatives
 - ➤1% fish alternatives
 - >2% other (eg egg alternatives / vegan desserts)
- Packaging:
 - >80% pre-packed, unopened
 - >3% pre-packed but opened
 - ▶10% loose / not pre-packed

Sampling point:

- 90% retail
- > 9% producers
- 1% catering

Results

- 92% satisfactory
- 3% borderline
- 5% unsatisfactory
 - > due to Enterobacteriaceae and E. coli
- No Salmonella detected
- Bacillus cereus borderline in 2 samples
- *L. monocytogenes* in 5 samples
- Other Listeria species in 4 samples



Satisfactory Borderline Unsatisfactory

Interpretation of Enterobacteriacae

- Are high Enterobacteriaceae levels expected in plant-based foods?
- Many products include a pasteurisation or cooking stage
- Borderline / unsatisfactory Enterobacteriaceae levels in: >17% of unpackaged or open packs
 5% of unopened packs
- Considered that it is reasonable to interpret Entero levels according to HPA / UKHSA Ready-To-Eat Guidelines

<100 cfu/g = satisfactory 100 – 10,000 cfu/g = borderline >10,000 cfu/g = unsatisfactory

Listeria in vegan products

• *L. monocytogenes* detected in 5 tofu samples from same producer:

Sampling date	No. samples	Product	L. mono Result	Туре
Jan 23	1	Organic natural tofu	Detected 20 cfu/g	Serotype 1/2a (ST37)
Feb 23 (early)	5	Organic natural tofu	3 x detected: 20, <20, <20 cfu/g	Serotype 1/2a (ST37) Serotype 4 (ST145)
Feb 23 (late)	5	Various tofu products	All negative	
Mar 23	5	Various tofu products	1 x detected 20 cfu/g	Serotype 1/2a (ST37)
Mar 23	1	Swab from producer	Detected	Serotype 1/2a (ST37)
May 23	1	Environmental sample – commercial lab		Serotype 4 (ST145)

• L. species in 4 meat substitutes (2 burgers / 2 'chicken')

Interpretation of Listeria results – EC 2073/2005

Chapter 1. Food safety criteria

Food category	Micro-organisms/their toxins, metabolites	Sampling plan (1)		Limits (2)		Analytical reference	Stage where the criterion applies
		n	с	m	М	method (3)	stage where the criterion applies
 Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes (⁴) 	Listeria monocytogenes	10	0	Absence	in 25 g	EN/ISO 11290-1	Products placed on the market during their shelf-life
1.2 Ready-to-eat foods able to support the growth of L monocytogenes, other than those intended for infants and for special medical purposes	Listeria monocytogenes	5	0	100 cfu/g (⁵)		EN/ISO 11290-2 (%)	Products placed on the market during their shelf-life
		5	0	Absence i	in 25 g(⁷)	EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
1.3 Ready-to-eat foods unable to support the growth of L monocytogenes, other than those intended for infants and for special medical purposes (4) (8)	Listeria monocytogenes	5	0	100	cfu/g	EN/ISO 11290-2 (%)	Products placed on the market during their shelf-life

'Unable to support growth'

Products with

- pH ≤ 4.4
- aw ≤ 0.92
- pH ≤ 5.0 AND aw ≤ 0.94
- shelf-life of less than five days

shall be automatically considered to belong to this category.

Other categories of products can also belong to this category, subject to scientific justification.

Impact of pH and water activity

- pH and Aw determined (Aw stopped from Feb 2023)
 - 38% had pH <5.0
 - 18% had Aw < 0.94
 - Mainly not protective against bacterial growth
- Tofu with *L. monocytogenes*: pH 5.3 – 6.3; Aw 0.97 – not protective
- Camembert-style cheese with 2800 cfu/g *B. cereus*: pH 5.2; Aw 0.95 – not protective
- Garlic and herb soft cheese with 8800 cfu/g *B. cereus*: pH 4.4 (Aw not determined) – control of *B. cereus* growth likely

Conclusions

- Plant-based meat/dairy alternatives are largely of a satisfactory microbiological quality
- Conditions allow *Listeria* survival and growth particularly in meat substitutes?
- Bacillus cereus not observed frequently but pH / Aw may not be sufficient to control growth when present
- Storage temperature and shelf-life must be carefully considered for these products to ensure safety
- Important that the public and Food Businesses don't assume these products are risk-free, just because they are plant-based

Acknowledgements

- FW&E teams at London, Porton and York
- Lorraine Sadler-Reeves study coordination
- Cat Startin data analysis and literature review

Publication:

Willis et al (2024) Journal of Applied Microbiology 135: Ixae245

rnal of Applied Microbio logy, 2024, 135(10), lxae24 https://doi.org/10.1093/jambio/lxae245 Advance access publication date: 26 September 2024 JOURNAL OF APPLIED Research Article OXFORD MICROBIOLOGY UNIVERSITY PRESS Microbiological quality of vegan alternatives to dairy and meat products in England during 2022-3 Caroline Willis^{1,*}, Catherine Startin¹, Frieda Jorgensen¹, Lorraine Sadler-Reeves¹, Heather Aird², ¹UK Health Security Agency, Food Water and Environmental Microbiology Laboratory Porton, Porton Down, Salisbury SP4 0JG, United Kingaom ²UK Health Security Agency, Food Water and Environmental Microbiology Laboratory York, York Biotech Campus, York YO41 1LZ, United Auguoun ³UK Health Security Agency, Food Water and Environmental Microbiology Services, Colindale, London NW9 5EQ, United Kingdom ⁴UK Health Security Agency, Postscietartinal Postaria Palaraana Unit, National Infantian Service, 51 Colindale August, London M 4 W Health Security Agency, Gastrointestinal Bacteria Reference Unit, National Infection Service, 61 Colindale Avenue, London NW9 5EQ, *Corresponding author. UK Health Security Agency, Food Water and Environmental Microbiology Laboratory Porton, Porton Down, Salisbury SP4 0JG, United Abstract Aims: Plant-based alternatives to meat and dairy products have become increasingly popular in the UK. Despite a public perception that they have a solution to the transmission of allower base linked with those foods. This strate simulate second the misrobiological second and the Hants, reincrussed and name or mean and dany products neve become increasingly popular in the on-bespree a popular perception data are have a relatively low microbiological risk, outbreaks of illness have been linked with these foods. This study aimed to assess the microbiological have a relatively low microbiological risk, outbreaks of illness have been linked with these foods. This study aimed to assess the microbiological interval and the second s Methods and results: Samples were collected between September 2022 and March 2023 from retail, production, and catering premises, and memory and resurts, panipes were concrete between September 2022 and march 2023 from retail, production, and catering premises, and tested for a range of bacterial pathogens and hygiene indicators using standard procedures. A total of 937 samples were tested, of which one indicators and 5% types to additional and 5% types types types to additional and 5% types typ tested on a range or pacterial parrogens and tryperie instructions using stativatio procedures. A total or bor samples were usated, or writeri 92% were of a satisfactory microbiological quality, 3% were borderline, and 5% were unsatisfactory. Those interpreted as unsatisfactory were does to abust of points of Entrophysicitations and Entrophysics of from the abust of the total of the theory of the total of at a verse of a demander y increasing our years, one verse water increasing on the second state of the sec monocytogenes was present in five samples of tofu, all from the same producer (all at counts of <100 CFUg-1), while other Listeria species monocytogenes was present in two samples or toru, as monitore same producer tail as counts or < 100 Cr Og 1/, while outer clasteral species was detected at counts of <20 CFU g⁻¹ in two burgers and two 'vegan chicken' products. The majority of samples did not have pH and water was burgers to the transition of <20 CFU g⁻¹ in two burgers and two 'vegan chicken' products. The majority of samples did not have pH and water was burgers to the transition of <20 CFU g⁻¹ in two burgers and two 'vegan chicken' products. 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Conclusions: The majority of vegan products examined were of a satisfactory quality, but results demonstrate that microbiological control must be maintained using appropriate processing and storage temperatures, and application of a safe length of shelf life. This study is one of the first to assess the hygiene and microbiological safety of vegan alternatives to meat and dairy products. While results Were largely satisfactory, it is important that producers and retailers understand the appropriate control measures to maintain safety throughout where largely satisfactory, it is important that producers and retailers understand the appropriate control measures to maintain safety throughout where large Keywords: vegan; plant-based; microbiological quality; Bacillus cereus; coagulase-positive staphylococci; E. col; Enterobacteriaceae; Listeria; Salmonella Introduction Plant-based foods consumed as alternatives to meat and dairy (2015) produced a risk ranking model for pathogens in ready products have become increasingly popular in the UK in reto eat, non-processed foods of non-animal origin, which idencent years. Consumers are choosing plant-based alternatives tified a strong risk for Shigella in fresh pods, legumes, and for environmental, lifestyle, and health reasons. A third of grains, and moderate risks for Salmonella in nuts and nut British meat eaters reported reducing their meat consumption products as well as Shiga-toxin-producing Escherichia coli in July 2018, and sales of meat-free foods grew by 40% be-(STEC) and Staphylococcus aureus in fresh pods, legumes, and tween 2014 and 2019 (Mintel 2020). In 2019, it was reported that the UK had overtaken Germany as the nation with the Vegan alternatives to milk of animal origin are commonly highest number of new vegan food products launched (Mintel produced by soaking nuts, grains, or pulses in water, while cheese alternatives may be made by soaking nuts, with or While products of animal origin tend to be considered without a subsequent fermentation stage. In 2021, a Brie-style higher risk than plant-based products in terms of microbicashew nut cheese caused an outbreak of Salmonella Duisological safety (Dewey-Mattia et al. 2018, Piglowski 2019), burg, affecting 20 people in the USA (Lewis et al. 2023). An there have been reports of foodborne outbreaks associated outbreak of listeriosis in France in 2022 was linked to nutwith nuts (ECDC-EFSA 2020), seeds (EFSA 2011, Meinen et based cheese alternatives, and affected five people, includal. 2019), flour (Vasser et al. 2021), and other plant-based ing four pregnant women who delivered prematurely (Outproducts (Farakos and Frank 2014). Da Silva Felicio et al. break News Today 2023). An oat-based drink was linked with two reports of illness in 2022 (Food Safety News 2022) Received 19 July 2024; revised 21 August 2024; accepted 25 September 2024 © The Author(s) 2024, Published by Oxford University Press on behalf of Applied Microbiology International. 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