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# Investigation of the growth of *Listeria* in plant-based beverages

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## ABSTRACT

The objective of the present study was to evaluate whether the content of sugar, protein, fat, or fibre in commercially available and specially formulated plant-based beverages (oat, soya and pea) influences the growth rates of *Listeria*. Beverages were inoculated with a strain cocktail of *Listeria* (approximately  $1 \times 10^3$  CFU/mL), and the data demonstrated that *Listeria* could proliferate in all tested beverages. Moreover, varying concentrations of naturally occurring or added sugar (0–3.3%), protein (3.3–5%), fat (1.1–3.5%) and added fibre (0–1.5%) did not have a statistically significant ( $p > 0.05$ ) impact on the growth rates of *Listeria* in the tested plant-based beverages. These data suggest that the wide variety of commercial plant-based beverages serve as an ideal medium for the growth of *Listeria* irrespective of product composition. All the various products tested provided sufficient nutrients to support at least a 2.6-log increase of *Listeria* within 16 h at room temperature, with some beverages supporting a 3-log increase. Therefore, these data highlight the importance of careful storage and handling of these increasingly varied and popular products.

## 1. Introduction

Plant-based milks are among the fastest-growing product categories in the global food market. Within Europe, sales for plant-based products climbed by 49% from 2018 to 2020, reaching €3.6 billion (Anon, 2021), and economic predictions show that the market will be worth €7.5 billion by 2025 (ING, 2020) and even \$16.7 billion by 2029 (Meticulous Research, 2022). Plant-based beverages are aqueous plant extracts that resemble animal milk in consistency and appearance. Most commercial plant-based beverages undergo ultra-high temperature (UHT) treatment. UHT-treated beverages are microbiologically safe, but post-opening contamination with pathogenic or spoilage bacteria is possible. The raw materials used for beverage production can be contaminated with foodborne pathogens such as *Listeria monocytogenes*, posing a food safety risk. There are documented recalls of *Listeria*-contaminated nuts, nut butter, and coconut products (FDA, 2020a, 2020b, 2020c, 2020d, 2019a, 2019b, 2018). *Listeria* was also found in soybeans and sprouts, and due to possible *Listeria* risk, oats cereals and granola products were recalled (FDA, 2023, 2021, 2017a, 2017b; Israel Ministry of Health, 2023).

Plant-based milk alternatives can significantly differ in composition values and nutrition profile according to their raw materials and formulations (Gobbi et al., 2019; Drownowski et al., 2021). In a study of the

nutritional composition of plant-based beverages, Walther et al. (2022) found that sucrose and glucose were the main carbohydrates present. However, to sweeten the beverages and improve palatability, white beet sugar or cane sugar (sucrose), glucose-fructose syrup, agave syrup (fructose), corn syrup (glucose) or stevia-derived sweeteners can be added. In some plant-based beverages, sugar content is modified using an enzymatic digestion step during production. U.S Food and Drug Administration (FDA) guidance on sugar labelling currently states that sugars created through the controlled hydrolysis of complex carbohydrates (e.g. starch) during production of plant-based beverages need to be declared as 'Added Sugars' on the Nutrition Facts label (FDA, 2019c). It is unclear whether manufacturers in non-U.S. countries comply with this labelling requirement. Some oat-based beverages are legally advertised as 'No Sugar' or 'Zero Sugar', as a different production process is followed, which does not result in the formation of sugars in situ. Some brands manufacture oat milk using oat protein only (i.e. without oat carbohydrates). Plant-based beverages also vary significantly in fat and protein concentrations. Reported fat concentrations generally resemble that available in bovine milk, i.e., low-fat (1.0/100 g), semi-skimmed (1.8/100 g), and whole-fat (3.5/100 g). Regarding protein, it should be noted that plant proteins differ from bovine proteins, and the amino acids present depend on the type of beverage and its raw ingredients. Many foods are at risk of *Listeria* contamination and are

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known to support this pathogen's growth. In a previous study, we highlighted that other plant-based beverages (coconut, almond and cashew) supported growth at various temperatures (4°C, 8°C, 20°C) and that the plant-based milk may represent an ideal medium for bacterial growth (Bartula et al., 2023). It has also been documented that soya milk supports *Listeria* growth (Ferguson and Shelef, 1990; Liu and Lin, 2008).

The effect of added sugars on *Listeria* growth in bovine milk has been reported by Rosenow and Marth (1987a), who observed that including additional sucrose (5.8%) had no significant impact on the generation times for *Listeria* at 13°C. Conversely, Ariahu et al. (2010) reported that increased sucrose concentration increased bacterial growth rate in soya milk. Lobete et al. (2017) also assessed the effect of table sugar, laboratory sucrose, commercial stevia and steviol glycosides on the growth dynamics of *Listeria* in soya milk and found that at 8°C and 20°C; there were no significant differences. We previously reported a significant difference in *Listeria* growth rates between different brands of almond plant-based beverages. Faster growth rates were observed in almond beverages where sugar, protein, and fibre content were higher, indicating that the addition of sugar in these products could potentially influence *Listeria* growth. However, no statistically significant difference in growth rates was observed in coconut beverages with varying sugar concentrations (Bartula et al., 2023). Currently, plant-based beverages typically contain low fibre concentrations. In the US, the fibre content is less than 0.5/100 g (Drewnowski et al., 2021), while some European products contain 1.5 g/100 mL. Although fibre is not a top focus of plant-based beverages, its inclusion could provide additional health benefits. Alginates, polysaccharides produced by brown seaweeds and algae, are often used in foods as a stabiliser and a source of dietary fibre (Brownlee et al., 2005), which can help manage health issues, including diabetes, colon cancer and bowel movements (Barak and Mudgil, 2014). However, locust bean gum is one of the most promising soluble dietary fibre sources (Ravat et al., 2019). It is obtained from natural sources (seed endosperm of *Ceratonia siliqua*), and its solutions are stable at a wide range of pH, which is a desirable property, especially in beverages. There has been little literature discussing the effect of fibre content on the growth dynamics of *Listeria*. Aspridou et al. (2014) investigated the growth of the pathogen using gelling agents, and it was reported that gelling agents can alter the growth rate of *Listeria*. However, this effect depended on the temperature and presence of glucose.

Growth data for *Listeria* in the wide range of plant-based beverages with varying added sugar, protein, fat and fibre concentrations would provide vital information for risk assessment of the pathogen in plant-based beverages and cast a new light on whether the concentrations of these ingredients significantly affect its growth. In addition, growth in these beverages would provide fundamental insight into the adaptability of this important pathogen in alternative plant-based products that are becoming more widespread. Therefore, in the current study, the impact of varying sugar, protein, fat and fibre concentrations in plant-based milk beverages was examined in detail.

## 2. Materials and methods

### 2.1. Source of samples and assessment of sterility

Non-refrigerated ultra-high temperature (UHT) treated oat and soya beverage samples were procured from local supermarkets (Cork, Ireland). Pasteurised and refrigerated pea beverages were specially formulated at the School of Food and Nutritional Sciences, University College of Cork (UCC, Cork, Ireland). To assess the presence of microbial contamination, 100 µl of each beverage was spread-plated on Brain Heart Infusion agar (BHI, Neogen, UK). Pasteurised products were additionally spread-plated on Mannitol egg Yolk Polymyxin agar (MYP, Neogen, UK). Plates were incubated at 37°C or 30°C for 24–48 h for BHI plates or MYP plates, respectively.

### 2.2. Production of pea beverages

Beverages were produced as described by Boeck et al. (2021) with some modifications. Briefly, 4.23% (w/w) pea protein isolate (Naturz Organics, Helmond, Netherlands), 1.25% (w/w) sucrose (Sigma-Aldrich, St. Louis, MO, USA), and 0.5–1.5% locust bean gum (Cargill, Minnesota, USA) or sodium alginate (Chemcolloids Ltd, Congleton, UK) were mixed with water and hydrated at 50°C for 1 h with brief mixing (160 rpm) in a stirring water bath (LB 8, Lochner Labor + Technik GmbH, Berching, Germany). 1.5% (w/w) sunflower oil was added to the solution, followed by shearing with an Ultra-Torrax T18 equipped with an S18N-19G dispersing element (IKA-Werke GmbH, Staufen, Germany) for 10 min at 6000 rpm. The solution was pasteurised at 85°C for 2 min in a stirring water bath, filled into sterile containers, and stored at 4°C. Beverages which did not contain locust bean gum or sodium alginate were used as controls.

### 2.3. Test Organisms and preparation of inoculum

The *Listeria* strains selected for this study were strains commonly used in food safety studies. *Listeria monocytogenes* (LO28, F2365, 10403S, and EGDe), and *Listeria innocua* (5072) were obtained from the Munster Technological University culture collection (MTU, Cork, Ireland). All strains were preserved in cryovials containing 40% glycerol in BHI broth (Neogen, UK) and stored at –20°C and –80°C. The bacterial strains were individually streaked on BHI agar.

### 2.4. Bacterial cocktail preparation and sample inoculation

For each *Listeria* strain, a single colony was used to inoculate 10 mL of BHI broth. All strains were cultivated individually overnight (16–18 h), with agitation at 20°C. Each overnight was centrifuged at 5000×g at 4°C for 5 min; the supernatant was discarded, and the collected pellet was washed with sterile Ringers' solution (1/4 strength/Merck), centrifuged as previously described and resuspended in 10 mL of sterile Ringers' solution. The cultures were individually standardised to 0.5 McFarland standard (~1 × 10<sup>8</sup> CFU/mL). To prepare a cocktail of *Listeria* strains, 1 mL aliquots of standardised cultures were combined in a test tube and vortexed to ensure homogeneity. The cocktail was serially diluted in sterile Ringers' solution to obtain a cell population of ~1 × 10<sup>5</sup> CFU/mL. For the UHT-treated products, 9.9 mL of each beverage was inoculated with 100 µl of the cocktail, resulting in an initial population of 10<sup>3</sup> CFU/mL. For the pasteurised beverages with varying fibre content, 59.4 g of each beverage was inoculated with 600 µl of the cocktail, resulting in a starting concentration of 10<sup>3</sup> CFU/mL.

### 2.5. Experimental conditions and sampling

Immediately after inoculation, 100 µl of undiluted and serially diluted samples (in sterile Ringers') were spread-plated on BHI (T = 0). Inoculated beverages were kept at ambient (20°C) temperatures for the remainder of the experiments. For all subsequent time points, 10 µl samples were serially diluted and spotted on BHI agar. Plates were incubated at 37°C for 24h before CFU counts. Uninoculated samples served as negative controls and were stored at experimental conditions. At the final timepoint, 100 µl of each negative control was plated on BHI/MYP agar, incubated at 37°C/30°C for 48h and examined for signs of microbial growth. A temperature recorder (LogTag®) was used to monitor temperatures every hour for the duration of the study.

### 2.6. Data analysis

All experiments were carried in biological triplicate. Growth rates were calculated using the DMFit provided by ComBase (<https://browser.combase.cc/DMFit.aspx>). Maximum growth rates were subjected to statistical analysis using GraphPad Prism Software, where data were

checked for statistically significant ( $p < 0.05$ ) differences through ANOVA and Tukey tests or Unpaired T-tests where appropriate.

### 3. Results

#### 3.1. Growth of *Listeria* in plant-based beverages with varying sugar content

The assays were carried out with the plant-based beverages that contain naturally occurring sugars produced in situ (oat beverage) or were additionally sweetened (soya beverage) (Table S1) and the appropriate 'No Sugar' labelled version, i.e., the version advertised to contain no sugars, either added or occurring naturally sugar from processing. The beverages were inoculated with a *Listeria*-cocktail, resulting in an initial population of  $\sim 10^3$  CFU/mL. The growth of the *Listeria*-cocktail was supported in all beverages at 20°C regardless of sugar content and type of beverage (Fig. 1). Despite differences in sugar contents, the overall maximum growth rates were not found to be significantly different ( $p > 0.05$ ) between the oat beverages containing 3.3 g of sugar and the 'No Sugar' version, and in 16 h, *Listeria* numbers were capable of increasing by 2.6–2.8 log CFU/mL. Similarly, no statistically significant difference ( $p > 0.05$ ) was observed between the soya beverage containing 2.5 g of sugar and the 'No Sugar' version, and in 16 h, *Listeria* numbers increased by 3.2–3.3 log CFU/mL. The growth rates appeared slightly higher in soya beverages than in oat beverages, but the difference was only significant ( $p < 0.05$ ) between the 'No Sugar' beverages.

#### 3.2. Growth of *Listeria* in oat-based beverages with varying fat content

The growth of the *Listeria*-cocktail was supported in all oat-based beverages at 20°C regardless of fat content (1.1–3.5%) (Fig. 2). In all beverages, *Listeria* numbers increased by 2.6–2.8 log CFU/mL within 16 h, and in the first 24 h, bacterial numbers increased by  $\sim 3.2$  log CFU/mL. The difference in the overall maximum growth rates was not statistically significant ( $p > 0.05$ ).

#### 3.3. Growth of *Listeria* in soya-based beverages with varying protein content

The soya-based beverages differed in protein content (3.3–5 g). As with other plant-based beverages in this study, all soya-based beverages supported the growth of the *Listeria*-cocktail (Fig. 2). Within 16 h, *Listeria* numbers increased by over 3 log CFU/mL in the beverages. The *Listeria* strains' overall maximum growth rates were not significantly

different ( $p > 0.05$ ) between the beverages with different protein concentrations.

#### 3.4. Growth of *Listeria* in pea-based beverages with varying fibre content

For this experiment, a specially-formulated pea beverage was used. Two sources of standard food-grade fibres, locust bean gum and sodium alginate, were examined at four concentrations. The control contained 0.2 g/100 mL of naturally occurring fibre (0% added fibre), and the remaining samples contained added fibre of 0.5, 1, and 1.5%.

##### 3.4.1. Locust bean gum

Pea-based beverages with varying fibre concentrations (0–1.5 %) of locust bean gum origin, inoculated with the *Listeria*-cocktail, were stored at room temperature (20°C) (Fig. 2). The bacterial numbers increased  $\sim 3$ – $3.3$  log CFU/mL within 16 h and by 4–4.5 log CFU/mL in the first 24 h. The overall maximum growth rates between beverages with different added fibres were not found to be statistically significant ( $p > 0.05$ ).

##### 3.4.2. Sodium alginate

Pea-based beverages with varying concentrations of sodium alginate (0–1.5%) were inoculated with the *Listeria*-cocktail and stored at 20°C (Fig. 2). Bacterial numbers increased by 3–3.2 log CFU/mL within 16 h in all beverages, and no effect of fibre concentration was observed with the difference in the overall maximum growth rates being statistically insignificant ( $p > 0.05$ ).

### 4. Discussion

The data in this study confirmed that plant-based beverages, despite the variability of beverage composition, serve as an ideal medium for the growth of *Listeria* (Table 1). A previous study revealed that plant-based beverages (almond, coconut, and cashew) support *Listeria* growth, and the growth rates were higher than in full-fat bovine milk. Growth rate differences were not observed at 4°C, but a significant difference ( $p < 0.05$ ) was observed at 8°C and 20°C, with the most variation seen at 20°C (Bartula et al., 2023). UHT-treated plant-based beverages can be stored unopened at room temperature for up to 12 months, and there is no consensus on the length of post-opening storage of these beverages. Product labels advise the opened products to be stored at fridge temperatures for 5–10 days, but it is unknown how well consumers comply with this recommendation. To our knowledge, this report is the first to compare the behaviour of *Listeria* in plant-based beverages or any beverage featuring modifications of protein, sugars, fibre, and fat. Given the variation in the beverage composition in this study, it was surprising

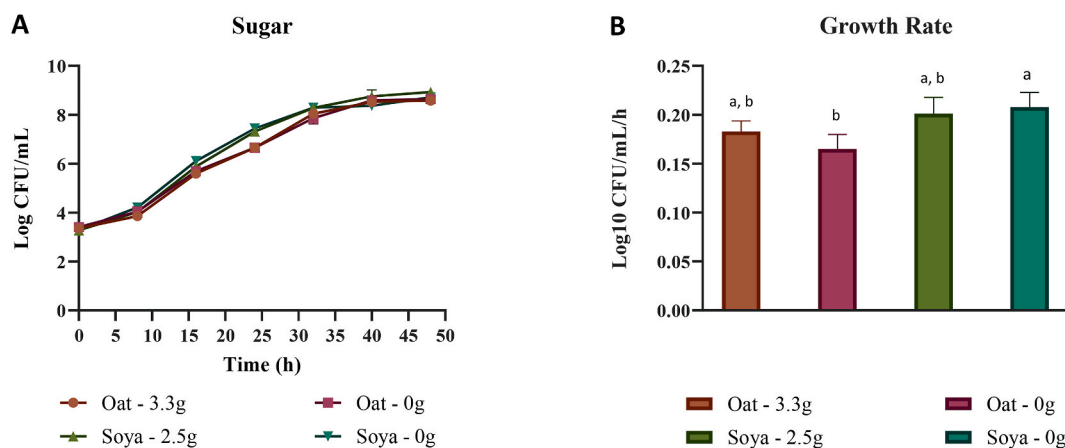
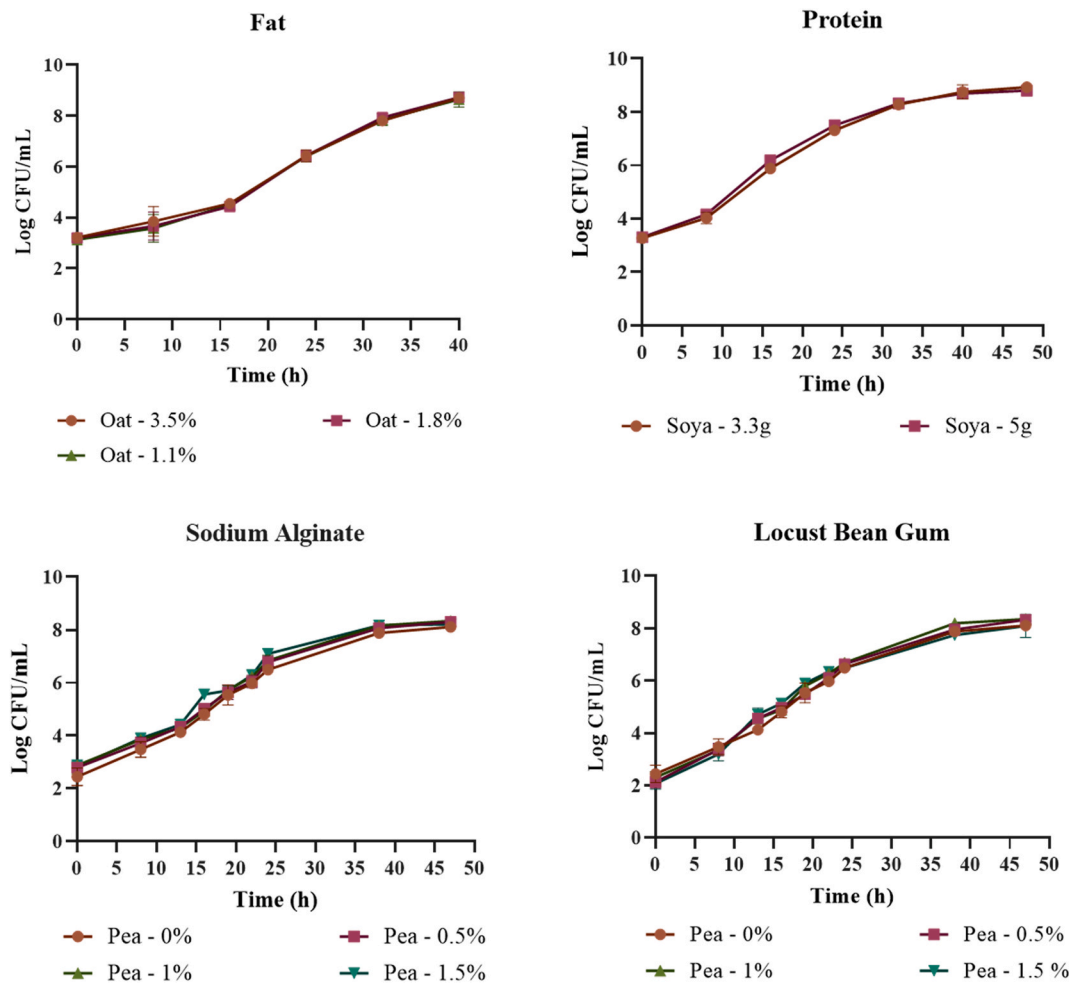


Fig. 1. Growth curve (A) and growth rates (B) of a cocktail of *Listeria* strains in oat and soya plant-based beverages with varying sugar content at 20°C. All data points represent a mean of triplicate results with  $\pm$  standard deviation. \* Sugar content (g) given is per 100 mL of the specified beverage. \*\* Maximum growth rates that do not share a letter significantly differ from their counterparts (95% confidence). Mean of triplicate results.



**Fig. 2.** Growth curves of a cocktail of *Listeria* strains in oat, soya, and pea plant-based beverages with varying concentrations of fat, protein, or fibre (sodium alginate or locust bean gum) at 20°C. Each time point on a growth curve represents a mean of triplicate results with  $\pm$  standard deviation. \* Varying gram and % levels refer to different formulas of the plant beverages (Table S1). Fat, protein and fibre content (g/%) given are per 100 mL of the specified beverage.

to see little variance in growth rates between most beverages. More importantly, all of the products tested provided sufficient nutrients to support at least a 2.6-log increase of *Listeria* within 16 h at room temperature and, in some products, a 3-log increase.

The available scientific literature suggests *Listeria* requires some amino acids for growth (Tsai and Hodgson, 2003; Joseph and Goebel, 2007; Sauer et al., 2019), which can be found in plant-based beverages at varying levels depending on beverage type (Walther et al., 2022). Increasing the protein level in soya-based beverages from 3.3% to 5% did not influence *Listeria* growth rates significantly ( $p > 0.05$ ). Of all plant-based beverages currently available, soya milk is considered the most nutritious in terms of protein content, as soy protein is composed of all essential amino acids required in the human diet (Bisla et al., 2012), and soya milk has a free amino acid concentration similar to bovine milk (Walther et al., 2022). The soya beverage with the lower protein level contained sufficient amino acid quantities to support *Listeria* growth, and increasing the protein concentration had no further impact on its growth. Plant-based beverages contain naturally occurring sugars that vary depending on the main ingredient and formulation process. They might also be additionally sweetened to improve the product's palatability. Sucrose and glucose are the main types of carbohydrates present in plant-based beverages (Walther et al., 2022), and oat milk can be high in maltose, depending on the production process. *Listeria* is known to be highly dependent on glucose ( $>0.5\%$ ) (Premaratne et al., 1991). It appears that in the Irish market, three product categories can be found in relation to sugar content. Products labelled as 'Added Sugar' – i.e.

additionally sweetened, 'No Added Sugar' – i.e. contain naturally-occurring or enzymatically-produced sugars, and 'No Sugar' or 'Zero Sugar' – i.e. carbohydrates are present, but 'sugars' were removed. There are discrepancies in the literature regarding the effect of the addition of sucrose on the growth dynamics of *Listeria*. In addition, Gilbreth et al. (2004) reported that in *Listeria*, the metabolism of arbutin, arabinol, cellobiose, mannose, maltose, trehalose, and salicin was repressed in the presence of glucose. Only fructose was metabolised along with glucose, and it is possible that catabolite repression by glucose influences growth rates in some beverages. However, there was no significant difference in the growth rates of *Listeria* in sweetened vs 'No Sugars' oat and soya drinks. The naturally occurring sugars from oats and soybeans must have been sufficient to support its growth. We only observed a statistically significant ( $p < 0.05$ ) difference in the growth rates between the 'No Sugars' versions of the oat and soya beverages.

The findings indicate that at 20°C, the change in the fat content (1.1–3.5%) in oat-based beverages did not significantly influence the growth rates of *Listeria*, which is consistent with the literature. One study found that the growth rate of *Listeria* was similar in skim, whole milk and whipping cream (Rosenow and Marth, 1987b). Similarly, variable fibre concentration (0–1.5%) did not influence the growth dynamics of *Listeria* in the pea-based beverages. This was true for both sodium alginate and locust bean gum. These results were generated in pasteurised rather than UHT beverages, but growth rates were similar in beverages subjected to the different heat treatments. In the same beverages, no



Table 1

Maximum growth rates of a cocktail of *Listeria* strains (log<sub>10</sub> CFU/mL/h, ±standard deviation) at 20°C in different plant-based beverages.

Beverage type:	Composition Factor <sup>b</sup> :	Maximum Growth Rate <sup>a</sup> :	R <sup>2</sup> value:	p-Value
<b>Sugar</b>				
Soya	0 g	0.208 ± 0.015 <sup>a</sup>	0.991	0.027
Soya	2.5 g	0.195 ± 0.018 <sup>a,b</sup>	0.993	†
Oat	0 g	0.165 ± 0.017 <sup>b</sup>	0.993	
Oat	3.3 g	0.183 ± 0.011 <sup>a,b</sup>	0.992	
<b>Fat</b>				
Oat	1.1 %	0.213 ± 0.028 <sup>a</sup>	0.996	0.400
Oat	1.8 %	0.193 ± 0.027 <sup>a</sup>	0.995	†
Oat	3.5 %	0.183 ± 0.022 <sup>a</sup>	0.993	
<b>Protein</b>				
Soya	3.3 g	0.195 ± 0.018 <sup>a</sup>	0.993	0.505
Soya	5 g	0.209 ± 0.009 <sup>a</sup>	0.990	‡
<b>Fibre – Locust Bean Gum</b>				
Pea	0 % <sup>c</sup>	0.201 ± 0.022 <sup>a</sup>	0.996	0.582
Pea	0.5 %	0.197 ± 0.008 <sup>a</sup>	0.995	†
Pea	1 %	0.213 ± 0.014 <sup>a</sup>	0.997	
Pea	1.5 %	0.210 ± 0.009 <sup>a</sup>	0.968	
<b>Fibre– Sodium Alginate</b>				
Pea	0 % <sup>c</sup>	0.201 ± 0.022 <sup>a</sup>	0.996	0.929
Pea	0.5 %	0.199 ± 0.008 <sup>a</sup>	0.994	†
Pea	1 %	0.207 ± 0.005 <sup>a</sup>	0.991	
Pea	1.5 %	0.204 ± 0.012 <sup>a</sup>	0.983	

† p-Values were obtained by comparing growth rates in an ANOVA test. Significance level  $\alpha = 0.05$ .

‡ p-Value was obtained by comparing growth rates in an Unpaired T-test. Significance level  $\alpha = 0.05$ .

<sup>o</sup> R<sup>2</sup> values indicate accuracy of model fitting for the growth rate calculation.

<sup>a</sup> Maximum growth rates that do not share a letter significantly differ from their counterparts (95% confidence). Mean of triplicate results.

<sup>b</sup> Varying gram and % levels refer to different formulas of the plant beverages (Table S1). Sugar, fat and fibre content (g/%) given are per 100 mL of the specified beverage.

<sup>c</sup> No additional fibre added. The beverage contained naturally contained fibre (0.2 g/100 mL).

difference in growth rate was observed at 4°C (unpublished data). *Listeria* can be found on raw materials used to produce plant-based beverages and might enter the product as a post-processing contaminant. Homemade plant-based milks, which are not typically subjected to heat treatment, are also becoming increasingly popular. Therefore, it is essential to highlight the need for sufficient heat treatment of homemade beverages.

## 5. Conclusion

In conclusion, this study highlights that plant-based beverages, despite the considerable variations in their nutritional composition, all constitute a rich growth medium and are vulnerable to *Listeria*-contamination. Variations of protein, sugar, fat and fibre in plant-based beverages had no significant influence on the growth dynamics of this foodborne pathogen.

## CRedit authorship contribution statement

**Klaudia Bartula:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft. **Sambou Biagui:** Investigation. **Máire Begley:** Supervision, Writing – review & editing. **Michael Callanan:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

## Declaration of competing interest

None.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2024.104530>.

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