Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.00479

Microbiological quality of home production of sprouts in Brazil

Vanessa dos Santos Chiappetta Nogueira SALGADO[1], Flávia Oliveira BRITO[1], Paula Stephany de Melo RODRIGUES[1], Adriane Elisabete Costa ANTUNES[2], Raquel GOUVÊA[1], Lilia ZAGO[1], Roberta Fontanive MIYAHIRA[1]*

Abstract

Sprouts are considered foods with high nutritional value, tasty, and easy to produce even in domestic environments. However, their consumption has been linked to various food outbreaks, as they are susceptible to microbial contamination. This work aimed to evaluate the microbiological quality of lentil, wheat, and chia sprouts germinated in a domestic environment and the effectiveness of the sprout and seed sanitization process. Total coliforms, *Escherichia coli*, mesophilic aerobes, and *Bacillus cereus* were determined, and the presence of *Salmonella* spp. and *Listeria monocytogenes* was investigated. No strains of *Salmonella* spp., *E. coli*, and *L. monocytogenes* were found. However, a high microbial load of total coliforms was found in the sprouts. *B. cereus* was found only in the wheat and chia sprout samples. The sanitization process with sodium hypochlorite was able to reduce the microbial load of the sprouts, but the load remained high. Seeds should be marketed only when they are suitable for home germination after going through a more efficient decontamination process. In addition, competent authorities are supposed to create and widely disseminate a document of good production practices for safer domestic cultivation of sprouts.

Keywords: bacteria; germination; seed disinfection; total coliforms; chemical decontamination.

Practical Application: Sprouts produced in a domestic environment showed a high microbial load, even after sanitization with sodium hypochlorite.

1 INTRODUCTION

Some countries, such as Brazil, the United States, and Australia, have nutritional guidelines that advocate that the consumption of fresh or minimally processed foods should be the basis for a nutritionally balanced, tasty, and sustainable diet, and this group includes foods such as grains, tubers, roots, vegetables, and fruits (Brasil, 2014; Australia, 2013; United States of America [USA], 2020). Owing to an increase in the number of individuals with chronic non-communicable diseases over the years, consumption of these foods is increasingly important, since a plant-based diet is a preventive factor for these diseases (Barros et al., 2021).

However, people's current lifestyle, characterized by a lack of time, is one of the main reasons for an inadequate diet (Menezes & Maldonado, 2015). Although cooking requires time and planning, it can contribute to better diet quality. Preparing and cooking one's own food helps the transition to and maintenance of a healthier lifestyle (Brasil, 2014; Oliveira & Castro, 2022).

Grains are staple foods that are widespread and consumed worldwide; they have an optimal nutritional composition and bring beneficial health effects (Aloo et al., 2021). Consumption of sprouts has become popular worldwide because consumers have been searching for foods with high nutrient content. The sprouting process leads to several changes in the nutritional composition of grains, e.g., it improves digestibility, increases nutritional value, reduces antinutritional factors, and favors the accessibility of nutrients (Miyahira et al., 2021). Peñas & Martínez-Villaluenga (2020) stated that germination reactivates seed metabolism, inducing the degradation of macronutrients and antinutritional compounds, and is also able to form bioactive compounds that are beneficial to health. Salgado et al. (2022) reported that an increase in protein content, dietary fiber, and phenolic compounds and a decrease in antinutritional factors occurred after germination in chia sprouts, for example (Salgado et al., 2022). In wheat sprouts, germination can increase antioxidant activity (Miyahira et al., 2022). In lentils, this process can increase the concentration of protein and nutrients such as iron, zinc, and manganese (Santos et al., 2020). Thus, the cultivation of sprouts in the home environment does not require a large space, and it consists of simple, economical, and sustainable steps. Moreover, it is an excellent strategy to encourage consumers to participate in the entire process of production of this highly nutritious food until the moment it is actually available for consumption (Ebert, 2022).

Received: Mar. 11, 2025.
Accepted: April 04, 2025.
[1]*Universidade do Estado do Rio de Janeiro, Institute of Nutrition, Rio de Janeiro, Rio de Janeiro, Brazil.*
[2]*Universidade Estadual de Campinas, School of Applied Sciences, Limeira, São Paulo, Brazil.*
*Corresponding author: robertamiyahira@gmail.com
Conflict of interest: nothing to declare.
Funding: This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Funding Code 001, and Foundation Carlos Chagas Filho Research Support of the State of Rio de Janeiro (FAPERJ) (project numbers E-26/211.830/2021 and E-26/210.919/2021).

Despite the demonstrated benefits of grain germination, the conditions of this process are favorable to the proliferation of pathogenic microorganisms present in the grain. Increased moisture, ambient temperature, and high nutrient availability result in an ideal scenario for microbial growth (Brankatschk et al., 2014; Ding et al., 2013; Iacumin & Comi, 2019). Foodborne outbreaks have been associated with sprout consumption in different parts of the world between 1988 and 2020, totaling about 15,342 cases, 313 hospitalizations, and 61 deaths (Carstens et al., 2019; Miyahira & Antunes, 2021).

In order to minimize the risks involved in sprout production, the U.S. Food and Drug Administration (FDA), along with the Institute for Food Safety and Health (IFSH), created the Sprout Safety Alliance (SSA), a public–private alliance that develops training programs and implementation of best practices for safe sprout production (SSA/FDA). Proper decontamination treatments should inactivate pathogenic microorganisms while preserving seed viability, germination, and vigor (National Advisory Committee on Microbiological Criteria for Foods [NACMCF], 1999). Although complete elimination of bacterial contamination by seed disinfection treatments in sprout production is very difficult to achieve (European Food Safety Authority [EFSA], 2011), studies have evaluated different methods (chemical, biological, and physical methods) of seed disinfection and the impact of these processes on germination capacity (Ding et al., 2013; Sikin et al., 2013). According to Gilbert et al. (2023), heat treatments that significantly reduce microbial growth also decrease seed germination capacity and are therefore not eligible for seed disinfection. Among the various chemical sanitizers recommended, sodium hypochlorite and hypochlorous acid were the most effective in reducing bacterial load by at least five logarithmic units without adversely affecting seed germination (Gilbert et al., 2023).

Considering that germination increases the nutritional value of grains, and germination conditions may be conducive to the development of pathogenic microorganisms, the microbiological contamination of sprouts and the efficiency of chemical sanitization of seeds and sprouts need to be evaluated for further development of actions to support regulations that ensure the safe production of sprouts. The reason is that sprouts are being increasingly grown in domestic environments and used in salads or food preparations that do not undergo any heat treatment for pathogen reduction. Thus, this study aimed to evaluate the microbiological quality of lentil, wheat, and chia sprouts produced in the home environment, as well as the effectiveness of the sanitization process with sodium hypochlorite on the sprouts and seeds.

1.1 Relevance of the work

Sprouting is a simple method that can be carried out at home, so this study aimed to evaluate the microbiological quality of sprouts produced in a domestic environment and the effectiveness of the sanitization process with sodium hypochlorite. High levels of total coliforms and mesophilic aerobic microorganisms were found. The microbial load remained high after sanitization with sodium hypochlorite. The results suggest the need to develop strategies for the safe production of sprouts, such as the marketing of seeds that have been treated and are suitable for germination and the dissemination of good sprout germination practices for home production.

2 MATERIALS AND METHODS

2.1 Samples and germination/sanitization process

Lentil (*Lens culinaris* L.), wheat (*Triticum aestivum* L.), and chia (*Salvia hispanica* L.) were divided into sanitized seed (SS) and unsanitized seed (USS) groups. After germination, the SS and USS groups were subdivided into four groups: unsanitized seed and unsanitized sprout (USS/USP), unsanitized seed and sanitized sprout (USS/SSP), sanitized seed and unsanitized sprout (SS/USP), and sanitized seed and sanitized sprout (SS/SSP). Figure 1 shows a sanitization/germination flowchart. The germination process was repeated five times for each of the four seed/sprout treatment groups, totaling 60 samples: 20 of lentil, 20 of wheat, and 20 of chia sprouts. Germination of lentil and wheat grains was performed according to Miyahira et al. (2022). The germination process of chia seeds was performed according to Abdel-Aty et al. (2021).

The sanitization process performed before (on the seeds) and after germination (on the sprouts) was the same. A solution of water (1 L) added to 10 mL of sodium hypochlorite at a concentration of 2% was prepared in a sterile container for 15 min (200 ppm), as specified on the product label by the manufacturer. Microbiological analyses were performed on the sprouts from March to October 2022, immediately after seed germination.

2.2 Microbiological analysis

2.2.1 Determination of total coliforms, Escherichia coli, mesophilic aerobic bacteria, and Bacillus cereus

Notably, 10 g of the sample was homogenized with 90 mL of 0.1% peptone water (10^{-1} dilution). Next, serial dilutions were made with 1 mL of the 10^{-1} dilution into a tube with 9 mL of 0.1% peptone water, performing the 10^{-2} dilution up to the 10^{-7} dilution.

Notably, 10 g of the sample was homogenized with 90 mL of 0.1% peptone water (10^{-1} dilution). Next, serial dilutions were made up to the 10^{-7} dilution (American Public Health Association [APHA], 2015). Total coliform and *E. coli* analyses were carried out using 3MTM PetrifilmTM plates. For mesophilic aerobic bacteria analysis, the pour plate technique was used, with plating of the dilutions on Standard Counting Agar (SCA) culture medium and incubation at 35°C ± 2°C for 48 h (APHA, 2015). For the determination of *B. cereus*, the spread plate technique was used as described by International Organization for Standardization (ISO) method 7932 (ISO, 2004).

2.2.2 Detection of Listeria monocytogenes

Notably, 25 g of the sample was homogenized in 225 mL of Buffered Listeria Enrichment Broth (BLEB) and incubated at 30°C/4 h (Hitchins et al., 2022). Selective agents were added

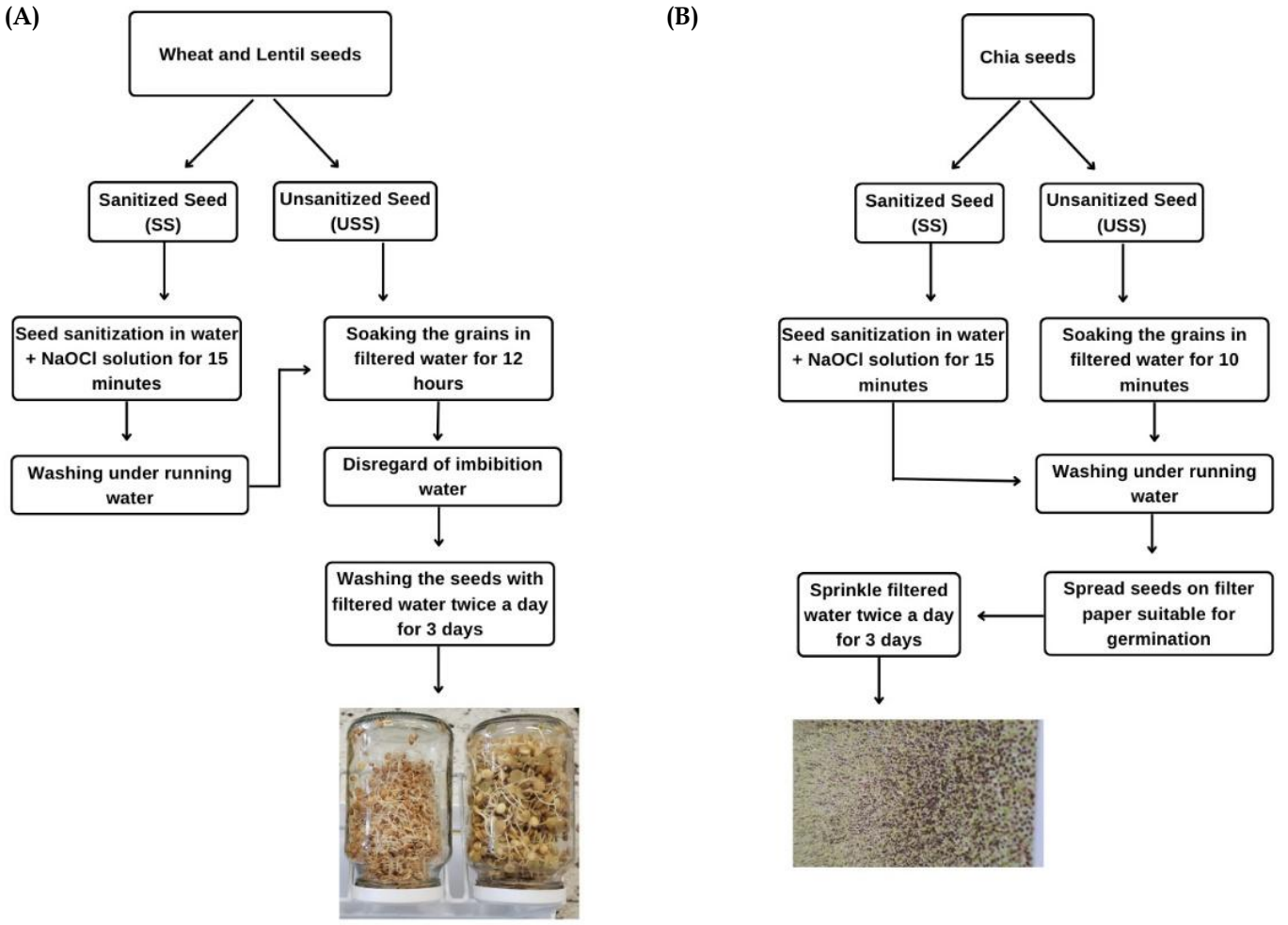

Figure 1. Flowchart of sanitization/germination of lentil, wheat (A), and chia (B) seeds.

and incubated at 30°C/44 h. After incubation, one well from the selective enrichment vial was streaked onto an Oxford Agar plate, and the plates were incubated at 35°C/24–48 h. When present, typical colonies were streaked on Soybean Trypticase Agar plates supplemented with 0.6% yeast extract and incubated at 30°C/24–48 h. For confirmation, the catalase, motility, and hemolysis tests were performed

2.2.3 Detection of Salmonella spp.

Notably, 25 g of the sample was homogenized in 225 mL of 1% peptone water and incubated at 37°C/24 h. Then, 1 mL of the pre-enriched sample was transferred to tubes with the selective media Tetrathionate Broth and Selenite Cystine Broth and incubated at 37°C/24 h. After that, one batch of each broth was streaked onto Hecktoen's Enteric Agar and Xylose Lysine Agar plates and incubated at 37°C/24 h. Typical colonies were inoculated into tubes slanted with Lysine Iron Agar and Triple Iron Sugar Agar for confirmation (Andrews & Hamamack, 2007).

2.3 MALDI-TOF

Typical isolated *Salmonella* spp. and *L. monocytogenes* colonies were identified by mass spectrometry (matrix-assisted laser desorption/ionization time-of-flight [MALDI-TOF]), according to the standard extraction protocol, using formic acid (Bruker, 2015), at the Medical Microbiology Research Laboratory of the Paulo de Góes Microbiology Institute at the Federal University of Rio de Janeiro.

2.4 Data analysis

The data collected from the microbiological analysis were analyzed and expressed in log CFU/g for total coliforms, *E. coli*, *B. cereus,* and total mesophilic aerobic bacteria, and in the presence/absence of *Salmonella* spp. and *L. monocytogenes* in 25 g of the sample. The results were presented as means and standard deviations calculated by Microsoft Excel. Analysis of variance (ANOVA) and Tukey's test were applied to determine statistically significant differences between the types of sanitization treatment, with a significance level of 5% ($p < .05$), using statistical software GraphPad Prism 9.

3 RESULTS AND DISCUSSION

The germination process is favorable to microbial growth, and some outbreaks involving sprouts have been reported in different parts of the world. The most recent were reported in

the year 2020, with 51 cases, with three hospitalizations, related to *E. coli* involving clover sprouts (Centers for Disease Control and Prevention [CDC], 2020), and in the year 2022, 63 people from eight states in the United States were infected with *Salmonella* Typhimurium strain after consuming raw alfalfa sprouts. It is estimated that the number of people infected in this latest outbreak was probably higher than reported because people recovered without medical care and without being tested for *Salmonella* (CDC, 2023).

A recent study showed that biohazard is not one of the main topics that consumers relate to sprouts. The study evaluated the perception of 315 Brazilian consumers about sprouts and showed that in a word association questionnaire, the "biological risk" was the least mentioned category. This result shows that few people related the consumption of sprouts with a negative aspect, such as foodborne diseases (FBD). This result shows that few people related the consumption of sprouts with a negative aspect, such as FBD (Miyahira et al., 2023). It is particularly relevant to assess the microbiological quality in the sprout production process to ensure consumption of a safe food, because dried seeds contain between 10^3 and 10^5 nonpathogenic bacteria per gram, which multiply rapidly during germination, and sprouts can contain between 10^8 and 10^9 nonpathogenic bacteria per gram until consumption (NACMCF, 1999). This very high bacterial population can make it difficult to detect pathogenic bacteria in sprouts. The location of bacteria on the surface of seeds/sprouts, their internalization potential, and their ability to adhere to seed/sprout tissues are also important factors that will influence the effectiveness of intervention strategies such as washing and decontamination (EFSA, 2011).

3.1 Total coliforms

In the present study, the results for total coliforms, in the lentil samples in which the sprouts were sanitized (USS/SSP), show that the values found were significantly lower than those in which the sprouts were not sanitized (USS/USP) (Table 1). In wheat, it was found that in the samples in which the sprouts had not been sanitized (USS/USP and SS/USP), there was a significantly higher microbial load of total coliforms than those that had been sanitized (USS/SSP and SS/SSP) (Table 1). For chia, it was found that the microbial load of total coliforms was significantly lower in the samples in which seeds and sprouts had been sanitized when compared to the samples in which the sprouts had not been sanitized, regardless of seed sanitization. It can be inferred that sanitization of the sprouts significantly reduced the number of total coliforms in the three types of study grains; however, it was found that the microbial load remained high, with values higher than 6.57 ± 1.65 log CFU/g. It is worth noting that when only the seed was sanitized, there was no significant reduction in the number of total coliforms in the three study sprouts.

Martínez-Villaluenga et al. (2008) conducted a study to evaluate the microbiological quality of broccoli and radish sprouts germinated for up to 5 days. Prior to germination, seeds were sanitized with 7% sodium hypochlorite for 30 min, washed and soaked with distilled water, and then placed in a seed germinator. Total coliforms were investigated, and an average value of 9.50 log CFU/g was found in broccoli seeds after germination. In the radish seeds, an average value of 8.04 log CFU/g was found. The values were similar to the one found in the present study, which is indicative of a high microbial load in the sprouts.

Table 1. Results of microbiological analyses of lentil, wheat, and chia sprouts before and after the seed and sprout sanitization process.

Sample	Microorganisms	USS/USP	USS/SSP	SS/USP	SS/SSP
		Mean ± SD			
Lentil	Total coliform (log CFU/g)	8.55 ± 0.09[a]	7.99 ± 0.32[b]	8.63 ± 0.02[a]	7.32 ± 0.13[c]
	Escherichia coli (log CFU/g)	< 1	< 1	< 1	< 1
	Aerobic mesophilic (log CFU/g)	8.88 ± 0.09[b]	8.17 ± 0.24[c]	9.38 ± 0.09[a]	8.41 ± 0.12[c]
	Bacillus cereus (log CFU/g)	< 2	< 2	< 2	< 2
	Listeria monocytogenes/25 g	Absence	Absence	Absence	Absence
	Salmonella spp./25 g	Absence	Absence	Absence	Absence
Wheat	Total coliform (log CFU/g)	8.41 ± 0.30[ab]	7.84 ± 0.05[c]	8.60 ± 0.31[a]	8.07 ± 0.12[bc]
	Escherichia coli (log CFU/g)	< 1	< 1	< 1	< 1
	Aerobic mesophilic (log CFU/g)	9.12 ± 0.10[a]	8.74 ± 0.25[a]	8.64 ± 0.41[a]	7.93 ± 0.48[b]
	Bacillus cereus (log CFU/g)	5.46 ± 0.51[ab]	4.88 ± 0.51[ab]	5.61 ± 0.62[a]	4.65 ± 0.47[b]
	Listeria monocytogenes/25 g	Absence	Absence	Absence	Absence
	Salmonella spp./25 g	Absence	Absence	Absence	Absence
Chia	Total coliform (log CFU/g)	8.51 ± 0.61[a]	7.59 ± 0.30[ab]	8.46 ± 0.14[ab]	6.58 ± 1.66[b]
	Escherichia coli (log CFU/g)	< 1	< 1	< 1	< 1
	Aerobic mesophilic (log CFU/g)	9.21 ± 0.26[a]	7.87 ± 0.71[ab]	8.73 ± 0.16[a]	6.58 ± 2.28[b]
	Bacillus cereus (log CFU/g)	6.03 ± 1.08[a]	5.90 ± 1.03a[b]	5.71 ± 0.67[ab]	4.22 ± 0.29[b]
	Listeria monocytogenes/25 g	Absence	Absence	Absence	Absence
	Salmonella spp./25 g	Absence	Absence	Absence	Absence

Caption: USS: unsanitized seed; SS: sanitized seed; USP: unsanitized sprout; SSP: sanitized sprout; SD: standard deviation; CFU: colony forming units; g: gram.
Identical letters in the same row indicate that there was no significant difference ($p < .05$) in the samples.

Another study by Tornuk et al. (2011) determined the number of total coliforms in wheat seeds and sprouts germinated for 9 days under different conditions of relative humidity (RH) (90 and 95%) and temperatures (18°C, 20°C, and 22°C) and investigated the disinfection capacity of sodium hypochlorite (NaOCl) (100, 200, and 400 ppm) and hydrogen peroxide (H_2O_2) (3 and 6%). The authors found a significant increase in total coliforms after germination and reported that increasing concentrations of NaOCl and H_2O_2 resulted in reductions in the total coliform population; there were greater reductions when seeds had been soaked in 400 ppm NaOCl for 30 min and then germinated at 18°C and 90% RH. It is worth noting that the authors also found that sodium hypochlorite at 200 ppm was also able to reduce total coliforms significantly by 0.54 log CFU/g. This result was different from that of the present study, which found no significant difference in the number of total coliforms after sanitizing only the seeds.

3.2 Escherichia coli

In this study, the presence of characteristic *E. coli* colonies was not found in any of the study samples. For this reason, it was not possible to evaluate the effectiveness of the sanitization process in reducing the amount of *E. coli* in the samples of this study. However, Tornuk et al. (2011), when analyzing the microbial load of wheat seeds and its changes during germination, found that there was an increase in the number of *E. coli* during germination in different conditions of humidity and temperature, reaching up to 6.37 log CFU/g at 90% humidity and 22°C temperature and 7.17 log CFU/g at 95% humidity and 22°C temperature. The authors also found that sanitization with sodium hypochlorite or hydrogen peroxide at different concentrations was able to significantly reduce the presence of this microorganism.

Jeddi et al. (2014) performed microbial evaluation of vegetables and sprouts of wheat and mung beans bagged in a supermarket chain and found the presence of *E. coli* in 12 of 64 sprout samples, corresponding to 18.7% of the study samples. Kim and Cheig (2021) evaluated the microbiological contamination of minimally processed products in Korea, and they found an average of 1 log CFU/g of *E. coli* in seven samples of mixed sprouts, which was considered to be within the acceptable limit according to the Korea Food Code standards. A literature review by Sikin et al. (2013) showed that several treatments are effective in decreasing the microbiological load of *E. coli* in seeds and sprouts; however, some sanitizers, such as organic acids, can impair the germination process or its sensory characteristics.

3.3 Aerobic mesophilic bacteria

The results of aerobic mesophilic (AM) in the lentil samples showed that the sanitized sprouts had statistically lower values than the unsanitized ones (Table 1), regardless of seed sanitization. Therefore, there was no reduction in the amount of AM when the seed was sanitized before sprouting, and the potential for AM internalization in lentil seeds and their structure may have influenced this result (EFSA, 2011). By contrast, in wheat, only SS/SSP had a significant reduction of AM when compared to the other samples (Table 1); i.e., the use of sanitization, only when performed simultaneously on the seeds and the sprouts, was effective in reducing the microbial load in wheat sprouts. In chia, sprout sanitization significantly reduced the amount of AM, while sanitization of the seeds alone was not able to reduce AM significantly (Table 1).

Saroj et al. (2006) reported that vegetable seeds can contain less than 2 log CFU/g of mesophilic aerobic bacteria and that this population of microorganisms can increase rapidly during germination owing to favorable conditions for bacterial growth. The mean AM count found in the present study was similar to the one found by Seow et al. (2012), who reported mean AM counts in bean sprouts sold in Singapore of 8.0 log CFU/g. The same occurred with the study conducted by Tango et al. (2018); the authors found the value of 9.35 log CFU/g of AM in mixed sprouts sold in Korea that had not been disinfected for microorganism reduction. Tornuk et al. (2011) compared the effectiveness of chemical sanitization in reducing AM and found that treating wheat seeds with 400 ppm NaOCl reduced AM count by 0.29 log compared to the control, and further found a 1.46 log reduction in sprouts after seed sanitization when compared to sprouts without seed sanitization. The reduction in the amount of AM in the study of Tornuk et al. (2011) was much greater than that found in wheat in the present study, which was 0.48 log between USS/USP and SS/USP samples. The authors explained that chlorine-based sanitizers affect microbial cells in various ways, e.g., by increasing membrane permeability, inhibiting enzyme systems, and irreversibly damaging the DNA of bacterial cells.

3.4 Bacillus cereus

Bacillus cereus (B. cereus) was isolated only in the wheat and chia sprouts because this bacterium is more present in cereals (Rahnama et al., 2023). The results (Table 1) showed that the SS/SSP wheat sample had a significant reduction of *B. cereus* only when compared to SS/USP. Thus, it can be suggested that sanitization of both seeds and sprouts is not able to reduce the microbial load of *B. cereus* adequately. In comparison, SS/SSP chia samples showed a significant reduction of *B. cereus* only when compared to USS/USP samples, suggesting that sanitization of the seeds only or the sprouts alone is not able to reduce contamination by *B. cereus*.

It is worth noting that this microorganism is involved in outbreaks of foodborne illnesses associated with vegetable products (Choi & Kim, 2020; Glasset et al., 2016; Ultee et al., 1999) and sprouts (Portnoy et al., 1976). Foodborne illnesses caused by *B. cereus* usually occur if the product has more than 5 log CFU/g (Harmon et al., 1987). Although the number of *B. cereus* cells required to produce sufficient emetic toxin to cause diseases is still unclear, studies have shown that levels of 10^3 to 10^{10} CFU/g have been found in foods involved in cases of emetic disease, and in most cases, there were at least 10^5 CFU/g (Arnesen et al., 2007; Dietrich et al., 2021). Thus, the unsanitized wheat and chia sprouts in the present study could be a risk for the development of FBD, as they showed values greater than 5 log CFU/g. In addition, the values of *B. cereus* found in the wheat and chia sprouts in the present study are above the criteria established

by the Brazilian legislation (Brasil, 2022) of 5×10^2 CFU/g (2.69 log CFU/g); therefore, wheat sprouts can be rated as unfit for consumption in Brazil.

Just like this study, the one by Pao et al. (2005) examined the growth of *Bacillus* spp. in sprouts grown in a domestic environment and found no growth of *B. cereus* during germination in lentil, alfalfa, and mung bean sprouts. However, in radish and broccoli sprouts, they found values close to 5 log CFU/g. In a study conducted in natural products stores in the Washington area, *B. cereus* was found to have values ≥ 3 MPN/g in 69% of 98 seed samples tested (

Harmon et al., 1987), with the average microbial load being 5.39 log CFU/g for wheat sprouts, as found in the present study.

3.5 Listeria monocytogenes

Although *L. monocytogenes* has been related to some food outbreaks with sprouts (CDC, 2015; Garner & Kathariou, 2016), this microorganism was not found in the sprouts in the present study, neither in the study by Abadias et al. (2008), who evaluated the microbiological quality of minimally processed fruits and vegetables and sprouts from retail establishments in Spain. These results were different from those reported by Seo et al. (2010), who made a microbial evaluation in 112 samples of sprouts (broccoli, alfalfa, soybean, and clover) produced in Seoul, Korea, and found the presence of this microorganism in one sample.

It is worth noting that these bacteria are difficult to isolate since microorganisms capable of growing on the selective enrichment medium used for recovery of *L. monocytogenes* to the levels required for detection may be present in the food analyzed, preventing the growth of *L. monocytogenes* through simple competition. In addition, the levels of *L. monocytogenes* in sprouts may be lower than the threshold required for direct detection and recovery, thus resulting in false negative test results (Cauchon et al., 2017).

Chlorine sanitization treatments appear to be promising in reducing *L. monocytogenes* in seeds and sprouts. Iacumin and Comi (2019) inoculated *L. monocytogenes* strains on mung bean seeds and demonstrated that the levels of *L. monocytogenes* can be reduced after four washes in chlorinated water solution (100 ppm) from 3.6 log CFU/g to < 10 CFU in seeds, and from 9.8 log CFU/g to 1.9 log CFU/g in sprouts. In addition, Lee et al. (2002) investigated the inhibition by chemical treatment of *L. monocytogenes* inoculated into mung bean sprouts purchased from commercial stores in Washington and showed that the use of sodium hypochlorite (200 ppm) for 10 min was able to reduce the amount of the bacteria by 1.02 log CFU/g. In another work, treatment with aqueous chlorine dioxide (100 ppm) for 5 min reduced the population of *L. monocytogenes* inoculated into mung bean sprouts by 1.5 log CFU/g (Jin & Lee, 2007).

3.6 Salmonella spp.

In the present work, no *Salmonella* spp. strains were isolated in any of the analyzed samples, and this result is considered satisfactory according to the Brazilian legislation (Brasil, 2022) and the European Commission regulation (European Commission, 2005), which determine that such strains must be absent. Similarly, Tornuk et al. (2011) investigated the presence of *Salmonella* spp. in wheat seed and sprouts, and Iacumin and Comi (2019) determined the quality of mung bean sprouts produced and sold in Italy between 2012 and 2016. Neither study found the presence of this microorganism.

However, in a trial aimed at evaluating the microbiological quality of 345 samples of minimally processed ready-to-eat vegetables, including 112 broccoli, alfalfa, soybean, and clover sprouts, Seo et al. (2010) found the presence of this microorganism in three samples of sprouts. In a survey by Saroj et al. (2006) that evaluated the microbiological quality of 124 sprouts marketed in India, *Salmonella* spp. was found in 24 samples. It is worth noting that, owing to the high risk of sprout-borne *Salmonella* infection, there is a need to improve the efficiency of detecting the presence of *Salmonella* spp. in sprouts, since this microorganism may be present at low concentrations or be sanitizer-injured (Zheng et al., 2015). Therefore, enrichment steps are considered as critical points in the detection of *Salmonella* spp. in sprouts, as they allow the rescue of cells of the bacterium to detectable levels (Zheng et al., 2015). Thus, in 2023, the FDA published an update of the Bacteriological Analytical Manual (Andrews et al., 2023), with new standards for the analysis of *Salmonella* spp. in sprouts, indicating the use of a specific broth for microbiological analysis of sprouts, the Universal Preenrichment Broth.

4 CONCLUSION

Disinfection with a household sanitizer reduced the microbial load of the sprout, especially as regards total coliforms and mesophilic aerobic bacteria. However, the microbial load remained high for both of them, which may have interfered with the possible detection of *L. monocytogenes* and *Salmonella* spp. Germination in the home environment seems to be a good strategy for the inclusion of a food item with high nutritional value in the diet; therefore, it is essential that seeds suitable for germination should be marketed.

In addition, national competent bodies need to create and disseminate a document of good production practices focused on the cultivation of sprouts, including in domestic environment, similar to the FDA—which is focused on industrial production—so that consumers can produce and safely consume this food at home from the microbiological point of view. Finally, further studies should be conducted to develop new sanitization techniques in the home environment that are effective in reducing the microbial load of sprouts as well as other fresh foods.

REFERENCES

Abadias, M., Usall, J., Anguera, M., Solsona, C., & Viñas, I. (2008). Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, *123*(1–2), 121–129. https://doi.org/10.1016/j.ijfoodmicro.2007.12.013

Abdel-Aty, A. M., Elsayed, A. M., Salah, H. A., Bassuiny, R. I., & Mohamed, S. A. (2021). Egyptian chia seeds (Salvia hispanica L.) during

germination: upgrading of phenolic profile, antioxidant, antibacterial properties and relevant enzymes activities. *Food Science Biotechnology*, *30*(5), 723–734. https://doi.org/10.1007/s10068-021-00902-2

Andrews, W. H., Wang, H., Jacobson, A., Ge, B., Zhang, G., & Hammack, T. (2023). Salmonella. In Food and Drug Administration (Ed.), *Bacteriological Analytical Manual* (April 2023 ed.). FDA. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella

Aloo, S. O., Ofosu, F. K., Kilonzi, S. M., Shabbir, U., & Oh, D. H. (2021). Edible Plant Sprouts: Health Benefits, Trends, and Opportunities for Novel Exploration. *Nutrients*, *13*(8), Article 2882. https://doi.org/10.3390/nu13082882.

American Public Health Association. (2015). *Compendium of Methods for the Microbiological Examination of Foods* (Y. Salfinger & M. L. Tortorello, Ed., 15th ed.). APHA.

Andrews, W. H., & Hamamack, T. (2007). Chapter 5: Salmonella. In United States Food and Drug Administration (Ed.). *Bacteriological Analytical Manual* (8th ed., pp. 1–21). United States Food and Drug Administration. https://fda.report/media/79991/BAM--Chapter-5--Salmonella-%28version-December-2007%29.pdf

Arnesen, L. P. S., O'Sullivan, K., & Granum, P. E. (2007). Food poisoning potential *of Bacillus cereus* strains from Norwegian dairies. *International Journal of Food Microbiology*, *116*(2), 292–296. https://doi.org/10.1016/j.ijfoodmicro.2006.12.021

Australia (2013). *Eat For Health: Australian Dietary Guidelines Summary*. National Health and Medical Research Council.

Barros, D. M., Silva, A. P. F., Moura, D. F., Barros, M. V. C., Pereira, A. B. S., Melo, M. A., Silva, A. L. B., Rocha, T. A., Ferreira, S. A. O., Siqueira, T. T. A., Carvalho, M. F., Freitas, T. S., Leite, D. R. S., Melo, N. S., Alves, T. M., Barbosa, T. S. L., Santos, J. S. S., Costa, M. P., Diniz, M. A., & Fonte, R. A. B. (2021). The influence of food and nutritional transition on the increase in the prevalence of chronic non-communicable diseases. *Brazilian Journal of Development*, *7*(7), 74647–74664. https://doi.org/10.34117/bjdv7n7-579

Brankatschk, K., Kamber, T., Pothier, J. F., Duffy, B., & Smits, T. H. M. (2014). Transcriptional profile of *Salmonella* enterica subsp. enterica serovar Weltevreden during alfalfa sprout colonization. *Microbial Biotechnology*, *7*(6), 528–544. https://doi.org/10.1111/1751-7915.12104

Brasil (2014). *Guia Alimentar para a População Brasileira* (2nd ed.). Ministério da Saúde.

Brasil (2022). *Instrução normativa n° 161, de 1º de julho de 2022*. Estabelece os padrões microbiológicos dos alimentos. Diário Oficial da União. https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-161-de-1-de-julho-de-2022-413366880

Bruker. (2015). *Instructions for Use Bruker Guide to MALDI Sample Preparation*. Retrieved February 04, 2025, from https://www.bc.edu/content/dam/bc1/top-tier/research/VPR/research-facilities/8702557_IFU_Bruker_Guide_MALDI_Sample_Preparation_Revision_E.pdf

Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate Outbreaks of Foodborne Illness in the United States Associated with Fresh Produce From 2010 to 2017. *Frontiers in Microbiology*, *10*, Article 2667. https://doi.org/10.3389/fmicb.2019.02667

Cauchon, K. E., Hitchins, A. D., & Smiley, R. D. (2017). Comparison of *Listeria monocytogenes* recoveries from spiked mung bean sprouts by the enrichment methods of three regulatory agencies. *Food Microbiology*, *66*, 40–47. https://doi.org/10.1016/j.fm.2017.03.021

Centers for Disease Control and Prevention. (2015). *Wholesome Soy Products, Inc. Sprouts and Investigation of Human Listeriosis Cases (Final Update)*. Retrieved February 04, 2025, from https://www.cdc.gov/listeria/ outbreaks/bean-sprouts-11-14/index.html

Centers for Disease Control and Prevention. (2020). *E. coli Outbreak Linked to Clover Sprouts*. Retrieved February 04, 2025, from https://www.cdc.gov/ecoli/2020/o103h2-02-20/

Centers for Disease Control and Prevention. (2023). *Salmonella*. Retrieved February 04, 2025, from https://www.cdc.gov/salmonella/index.html

Choi, W., & Kim, S.-S. (2020). Outbreaks, germination, and inactivation of Bacillus cereus in food products: a review. *Journal of Food Protection*, *83*(9), 1480–1487. https://doi.org/10.4315/0362-028X.JFP-19-429

Dietrich, R., Jessberger, N., Ehling-Schulz, M., Märtlbauer, E., & Granum, P. E. (2021). The food poisoning toxins of Bacillus cereus. *Toxins*, *13*(2), Article 98. https://doi.org/10.3390/toxins13020098

Ding, H., Fu, T.-J., & Smith, M. A. (2013). Microbial contamination in sprouts: how effective is seed disinfection treatment? *Journal of Food Science*, *78*(4), R495–R501. https://doi.org/10.1111/1750-3841.12064

Ebert, A. W. (2022). Sprouts and microgreens—novel food sources for healthy diets. *Plants*, *11*(4), Article 571. https://doi.org/10.3390/plants11040571

European Commission. (2005). *Guidance Document on Listeria Monocytogenes shelf-life studies for ready-to-eat foods, under Regulation (EC) (No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs*. https://food.ec.europa.eu/system/files/2016-10/biosafety_fh_mc_guidance_document_lysteria.pdf

European Food Safety Authority. (2011). Scientific opinion on the risk posed by Shiga toxin-producing Escherichia coli (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA Journal*, *9*(11), Article 2424. https://doi.org/10.2903/j.efsa.2011.2424

Garner, D., & Kathariou, S. (2016). Fresh produce–associated listeriosis outbreaks, sources of concern, teachable moments, and insights. *Journal of Food Protection*, *79*(2), 337–344. https://doi.org/10.4315/0362-028X.JFP-15-387

Gilbert, G.S., Diaz, A., & Bregoff, H. A. (2023). Seed disinfestation practices to control seed-borne fungi and bacteria in home production of sprouts. *Foods*, *12*(4), Article 747. https://doi.org/10.3390/foods12040747

Glasset, B., Herbin, S., Guillier, L., Cadel-Six, S., Vignaud, M.-L., Grout, J., Pairaud, S., Michel, V., Hennekinne, J.-A., Ramarao, N., & Brisabois, A. (2016). Bacillus cereus-induced food-borne outbreaks in France, 2007 to 2014: epidemiology and genetic characterisation. *Eurosurveillance*, *21*(48), Article 30413. https://doi.org/10.2807/1560-7917.ES.2016.21.48.30413

Harmon, S. M., Kautter, D. A., & Solomon, H. M. (1987). Bacillus cereus contamination of seeds and vegetable sprouts grown in a home sprouting kit. *Journal of Food Protection*, *50*(1), 62–65. https://doi.org/10.4315/0362-028X-50.1.62

Hitchins A. D., Jinneman K., & Chen Y. (2022). Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods. In Food and Drug Administration (Ed.), *Bacteriological Analytical Manual* (April 2022 ed.). FDA. https://www.fda.gov/food/laboratory-methods--food/bam-chapter-10-detection-listeria-monocytogenes-foods--and-environmental-samples-and-enumeration

Iacumin, L., & Comi, G. (2019). Microbial quality of raw and ready--to-eat mung bean sprouts produced in Italy. *Food Microbiology*, *82*, 371–377. https://doi.org/10.1016/j.fm.2019.03.014

International Organization for Standardization. (2004). *International Standard ISO 7932. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presuntive Bacillus cereus - Colony count technique at 30°C* (3th ed.). International Organization for Standardization.

Jeddi, M. Z., Yunesian, M., Gorji, M. E., Noori, N., Pourmand, M. R., & Khaniki, G. R. J. (2014). Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *Journal of Health Population and Nutrition*, *32*(3), 391–399. https://pubmed.ncbi.nlm.nih.gov/25395902/

Jin, H.-H., & Lee, S.-Y. (2007). Combined effect of aqueous chlorine dioxide and modified atmosphere packaging on inhibiting Salmonella Typhimurium and Listeria monocytogenes in mungbean sprouts. *Journal of Food Science*, *72*(9), M441–M445. https://doi.org/10.1111/j.1750-3841.2007.00555.x

Kim, M.-J., & Cheigh, C.-I. (2021). Microbiological contamination of fresh-cut produce in Korea. *Food Science Biotechnology*, *31*(1), 79–87. https://doi.org/10.1007/s10068-021-01014-7

Lee, S.-Y., Yun, K.-M., Fellman, J., & Kang, D.-H. (2002). Inhibition of Salmonella Typhimurium and Listeria monocytogenes in mung bean sprouts by chemical treatment. *Journal of Food Protection*, *65*(7), 1088–1092. https://doi.org/10.4315/0362-028x-65.7.1088

Menezes, M. F. G., & Maldonado, L. A. (2015). Do nutricionismo à comida: a culinária como estratégia metodológica de educação alimentar e nutricional. *Revista Hospital Universitário Pedro Ernesto*, *14*(3), 82–89. https://doi.org/10.12957/rhupe.2015.19950

Martínez-Villaluenga, C., Frías, J., Gulewicz, P., Gulewicz, K., & Vidal-Valverde, C. (2008). Food safety evaluation of broccoli and radish sprouts. *Food and Chemical Toxicology*, *46*(5), 1635–1634. https://doi.org/10.1016/j.fct.2008.01.004

Miyahira, R. F., & Antunes, A. E. C. (2021). Bacteriological safety of sprouts: a brief review. *International Journal of Food Microbiology*, *352*, Article 109266. https://doi.org/10.1016/j.ijfoodmicro.2021.109266

Miyahira, R. F., Giangiarulo, T., Lopes, J. O., Silva, W. P., Esmerino, E. A., & Antunes, A. E. C. (2023). Sprouts consumption: A correlational study between Brazilian consumers' perception, motivation to eat new products levels and food choice factors. *Journal of Sensory Studies*, *38*(4), Article e12829. https://doi.org/10.1111/joss.12829

Miyahira, R. F., Lopes, J. O., & Antunes, A. E. C. (2021). The use of sprouts to improve the nutritional value of food products: a brief review. *Plant Foods of Human Nutrition*, *76*(2), 143–152. https://doi.org/10.1007/s11130-021-00888-6

Miyahira, R. F., Pena, F. L., Fabiano, G. A., Lopes, J. O., Ponte, L. G. S., Cunha, D. T., Bezerra, R. M. N., & Antunes, A. E. C. (2022). Changes in phenolic compound and antioxidant activity of germinated broccoli, wheat, and lentils during simulated gastrointestinal digestion. *Plant Foods of Human Nutrition*, *77*(2), 233–240. https://doi.org/10.1007/s11130-022-00970-7

National Advisory Committee on Microbiological Criteria for Foods. (1999). Microbiological safety evaluations and recommendations on sprouted seeds. *International Journal of Food Microbiology*, *52*(3), 123–153. https://doi.org/10.1016/s0168-1605(99)00135-x

Oliveira, M. F. B., & Castro, I. R. R. (2022). Cooking autonomy: a multilevel conceptual model on healthy home cooking. *Cadernos de Saúde Pública*, *38*(4), Article EN178221. https://doi.org/10.1590/0102-311XEN178221

Pao, S., Khalid, M. F., & Kalantari, A. (2005). Microbial profiles of on-line–procured sprouting seeds and potential hazards associated with enterotoxigenic bacillus spp. in homegrown sprouts. *Journal of Food Protection*, *68*(8), 1648–1653. https://doi.org/10.4315/0362-028x-68.8.1648

Peñas, E., & Martínez-Villaluenga, C. (2020). Advances in production, properties and applications of sprouted seeds. *Foods*, *9*(6), Article 790. https://doi.org/10.3390/foods9060790

Portnoy, B. L., Goepfert, J. M., & Harmon, S. M. (1976). An outbreak of Bacillus cereus food poisoning resulting from contaminated vegetable sprouts. *American Journal of Epidemiology*, *103*(6), 589–594. https://doi.org/10.1093/oxfordjournals.aje.a112263

Rahnama, H., Azari, R., Yousefi, M. H., Berizi, E., Mazloomi, S. M., Hosseinzadeh, S., Derakhshan, Z., Ferrante, M., & Conti, G. O. (2023). A systematic review and meta-analysis of the prevalence of Bacillus cereus in foods. *Food Control*, *143*, Article 109250. https://doi.org/10.1016/j.foodcont.2022.109250

Salgado, V. S. C. N., Zago, L., Antunes, A. E. C., & Miyahira, R. F. (2022). Chia (Salvia hispanica L.) Seed Germination: a Brief Review. *Plant Foods for Human Nutrition*, *77*(4), 485–494. https://doi.org/10.1007/s11130-022-01011-z

Santos, C. S., Silva, B., Valente, L. M. P., Gruber, S., & Vasconcelos, M. W. (2020). The effect of sprouting in lentil (Lens culinaris) nutritional and microbiological profile. *Foods*, *9*(4), Article 400. https://doi.org/10.3390/foods9040400

Saroj, S. D., Shashidhar, R., Dhokane, V., Hajare, S., Sharma, A., & Bandekar, J. R. (2006). Microbiological evaluation of sprouts marketed in Mumbai, India, and its suburbs. *J Journal of Food Protection*, *69*(10), 2515–2518. https://doi.org/10.4315/0362-028x-69.10.2515

Seo, Y.-H., Jang, J.-H., & Moon, K.-D. (2010). Microbial evaluation of minimally processed vegetables and sprouts produced in Seoul, Korea. *Food Science and Biotechnology*, *19*(5), 1283–1288. https://doi.org/10.1007/s10068-010-0183-y

Seow, J., Ágoston, R., Phua, L., & Yuk, H.-G. (2012). Microbiological quality of fresh vegetables and fruits sold in Singapore. *Food Control*, *25*(1), 39–44. https://doi.org/10.1016/j.foodcont.2011.10.017

Sikin, A. M., Zoellner, C., & Rizvi, S. S. H. (2013). Current Intervention Strategies for the Microbial Safety of Sprouts. *Journal of Food Protection*, *76*(12), 2099–2123. https://doi.org/10.4315/0362-028x.jfp-12-437

Tango, C. N., Wei, S., Khan, I., Hussain, M. S., Kounkeu, P.-F. N., Park, J.-H., Kim, S.-H., & Oh, D. H. (2018). Microbiological Quality and Safety of Fresh Fruits and Vegetables at Retail Levels in Korea. *Journal of Food Science*, *83*(2), 386–392. https://doi.org/10.1111/1750-3841.13992

Tornuk, F., Ozturk, I., Sagdic, O., & Yetim, H. (2011). Determination and Improvement of Microbial Safety of Wheat Sprouts with Chemical Sanitizers. *Foodborne Pathogens and Disease*, *8*(4), 503–508. https://doi.org/10.1089/fpd.2010.0709

Ultee, A., Kets, E. P., & Smid, E. J. (1999). Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. *Applied and Environmental Microbiology*, *65*(10), 4606–4610. https://doi.org/10.1128/AEM.65.10.4606-4610.1999

United States of America (2020). *Dietary Guidelines for Americans, 2020–2025* (9th ed.). United State Department of Agriculture; United State Department of Health and Human Services. https://www.dietaryguidelines.gov/resources/2020-2025-dietary-guidelines-online-materials

Zheng, Q., Mikš-Krajnik, M., D'Souza, C., Yang, Y., Heo, D.-J., Kim, S.-K., Lee, S.-C., & Yuk, H.-G. (2015). Growth of healthy and sanitizer-injured Salmonella cells on mung bean sprouts in different commercial enrichment broths. *Food Microbiology*, *52*, 159–168. https://doi.org/10.1016/j.fm.2015.07.013