



Microbial quality of raw and ready-to-eat mung bean sprouts produced in Italy



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ABSTRACT

The aim of this study was to determine the microbial quality of mung bean sprouts produced in Italy. The presence of pathogenic microorganisms (Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp. and *Listeria monocytogenes*), total coliforms, and total viable counts (TVCs) were determined. The study covered five years of sprout production. The results demonstrated that no pathogenic microorganisms were present, and the microbial load was less than 6 log CFU/g. The mung bean sprouts currently produced in Italy were found to be acceptable for consumption. An additional aim was to determine the fate of different strains of STEC, *L. monocytogenes* and *Salmonella* spp. by intentionally inoculating mung bean seeds during sprouting and by using chlorinated water to reduce the concentration of these strains in seeds and sprouts. The data demonstrated that these strains increased over 5–6 log CFU/g within 3 days from inocula. The chlorinated washing solution reduced the concentration of the investigated strains in seeds and sprouts by approximately 3 and 7 log CFU/g, respectively. However, it was not possible to completely eliminate the pathogens from either the mung bean seeds or sprouts. Despite these encouraging results, the producer's attention to hygienic quality should not be reduced when attempting to produce safe-to-consume mung bean sprouts.

1. Introduction

The use of mung bean sprouts, which originate from Far Eastern areas, has recently become widespread in the Western world. They are obtained from germinating seeds and are considered fashionable and healthy ingredients (Kuo et al., 2004). Mung bean sprouts are also recognized as fundamental for the human diet, as they provide health benefits as important sources of proteins, vitamins, minerals and fibre (Martínez-Villaluenga et al., 2008). The flow sheet of production requires that mung bean sprouts must be grown in environmentally controlled, warm (22–24 °C) and high humidity (95%) conditions for three to five days to sprout (Peles et al., 2012; Taormina et al., 1999a). Seeds are usually contaminated by high microbial loads, including yeasts, pseudomonads, enterobacteria and lactic acid bacteria. Due to the humid and nutritious sprouting conditions that are a good media for sprouts, the microbial loads can increase to a level of 10 log CFU/g (Zheng et al., 2015, 2016; Randazzo et al., 2009; Peñas et al., 2008; Ghandi and Matthews, 2003; Harris et al., 2003; Robertson et al., 2002; Prokopowich and Blank, 1991). Pathogenic microorganisms can also contaminate mung bean sprouts, either as primary contamination (while growing and harvesting) or secondary contamination (during washing, soaking, packaging and preparation), (Harris et al., 2003). The pathogen contamination of sprouts comes from the seeds. Consequently, it is widely recognized that sprout-associated outbreaks are due to seeds that are contaminated with pathogenic microorganisms

(NACMCF, 1999; Peles et al., 2012) rather than post-production contamination (Xiao et al., 2014). The pathogenic microorganism load increases because of the high volumes of water used during sprout production and due to the temperature for sprouting, particularly in systems where sprouts are exposed to a common “water bath” and frequently or continuously mixed, such as in rotary drums (Xiao et al., 2014). Sprouted seeds have thus been implicated in numerous and serious outbreaks caused by *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC). Indeed, *Salmonella enterica* serovar Bareilly in the UK (Cleary et al., 2010), *S. enterica* serovar Newport in Germany and the Netherlands (Bayer et al., 2014) and *S. enterica* serovar Enteritidis in the USA (FDA, 2014) caused outbreaks associated with bean sprouts. *Salmonella* and *Escherichia coli* O157:H7 are the most frequently associated causative agents of outbreaks caused by alfalfa and mung bean sprout consumption in North America (CDC, 2012; Health Canada, 2012; Fett, 2005), clearly indicating that mung bean sprouts constitute a significant food safety risk (Gómez-Aldapa et al., 2015). Numerous major STEC outbreaks have also occurred, particularly in northern Germany, in 14 other EU countries and in the USA and Canada, and some outbreaks were associated with the consumption of sprouted fenugreek seeds (EFSA, 2012; Health Canada, 2012; Buchholz, 2011). As sprouts pose a very complex challenge to bacterial pathogen enrichment and detection, the aim of this work is to determine the microbial quality of mung bean sprouts produced and sold in Italy. Additional aims were to determine the fate of different strains of STEC, *L.*

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monocytogenes and *Salmonella* spp. by intentionally inoculating mung bean seeds during sprouting and by using chlorinated water to reduce their concentrations in seeds and sprouts.

2. Material and methods

2.1. Media and sampling

Mung bean sprouts were collected from Italian supermarkets (300 lots) and directly from two sprout producers (60 lots) from January 1, 2012 to December 31, 2016. The lots included three replicate samples of approximately 250 g each and were produced by the two main Italian sprout producers, of which the first (a) produces approximately 90% and the second (b) produces approximately 10% of the mung bean sprouts sold in Italy. Sprouts were sampled and analysed after 1 day on the market, considering that their shelf life is approximately 5 days at a temperature less than 7 °C. In particular, 270 sprout lots produced by the (a) producer and 30 lots produced by the (b) producer were collected from 5 different supermarkets, 54 sprout lots were collected directly from the (a) producer and 6 lots from the (b) producer facility. Three hundred sixty mung bean seed samples of 15 lots, of which 9 lots were from the (a) producer and 6 lots were from the (b) producer, were also analysed during the same 5 years, using 6 samples for each month. Each sample included 3 replicates of 250 g each. Seeds all originated from Australia.

Each sample of sprouts or seeds was mixed in a stomacher bag, and 25 g of each sample was serially diluted with saline-peptone water (8 g/l NaCl, 1 g/l bacteriological peptone; Oxoid, Italy, distilled water 1000 ml) in stomacher bags.

An aliquot of 0.1 ml or 1 ml of each serial dilution was used to count different groups of microorganisms. Total viable count (TVC) was evaluated on plate count agar (Oxoid, Italy) incubated at 30 °C for 48–72 h. Total coliforms and *Escherichia coli* were detected by ISO 4832/2006 using two incubation temperatures (37 °C and 44 °C) and plated in a double layer.

The presence of pathogenic microorganisms was also detected in 25 g of enrichment culture based on the following methods: *Salmonella* spp. by the ISO 6579-1 2002; *Listeria monocytogenes* by ISO 11290-1, 2:1996 Adm. 1:2004 and STEC by Cocolin et al. (2000) during 2012 sampling year and then with ISO CEN TS 13136 method for the remaining sampling years.

2.2. Identification of coliforms isolated from sprouts

From the Violet Bile Lactose Agar plate (VRBLA) containing between 30 and 150 colonies of each sample, 3 colonies that were presumptive total coliforms were randomly isolated. Consequently, 180 colonies were collected and identified. The colonies were streaked on plate count agar (Oxoid, Italy) and then stored at –80 °C in brain heart infusion broth (Oxoid, Italy) supplemented with glycerol (30% final concentration, Sigma-Aldrich, Germany). The isolates were subjected to gram staining and oxidase and catalase tests and were then identified according to molecular method (PCR-DGGE and sequencing); briefly, each isolated bacterium was amplified with primers P1 and P4, as described by Klijn et al. (1991) and Iacumin et al. (2009), targeting 700 bp of the V1–V3 region of the 16S rRNA gene (rDNA). After purification, products were sent to a commercial facility for sequencing (Eurofins MWG GmbH, Martinsried, Germany). Sequences were aligned with those in GenBank with the BLAST program (Altschul et al., 1997) to determine the closest known relatives based on partial 16S rRNA gene homology.

2.3. Fate of the total viable count and total coliforms of mung bean sprouts during storage (4 ± 2 °C) for 5 days

Three lots produced by the (a) facility were sampled in March, April

and May of each investigated year from 2012 to 2016. Each lot included 20 samples and was analysed at day 0 (10 samples) and at 5 days (10 samples, end of shelf life) using the above method reported in section 2.1.

2.4. Bacterial strains and inoculum preparation

The bacteria strains included the following: *Listeria monocytogenes* isolated from meat and vegetables: *Listeria monocytogenes* Scott A, *L. monocytogenes* NCTC 10887 (serotype 1/2b), and *L. monocytogenes* NCTC 10527 (serotype 4b); *Salmonella* isolated from vegetables: *S. Enteritidis*, *S. Typhimurium* and *S. Derby*; *Escherichia coli* (STEC): *E. coli* O157:H7 strain ATCC 43888; *E. coli* O103:H2 ED-173 Istituto Superiore Sanità, Rome; and *E. coli* O26:H11 E-D 56, Istituto Superiore della Sanità, Rome. Each strain was grown in brain heart infusion agar (Oxoid, Italy). The inocula were obtained according to the modified method of Xiao et al. (2014). Four millilitres of an overnight culture in brain heart infusion broth (Oxoid, Italy) of each strain was subjected to centrifugation at 13,400 rpm for 10 min at room temperature, and the pellets were resuspended in saline-peptone water (8 g/l NaCl, 1 g/l bacteriological peptone; Oxoid, Italy, distilled water 1000 ml). The concentration of each suspension was approximately 6–7 log CFU/ml. Equal volumes of cell suspensions of each species were combined as a cocktail to obtain three different suspensions containing *L. monocytogenes*, *Salmonella* spp. and STEC with the desired cell concentrations for seed inoculation. Each suspension was diluted in saline-peptone water, and 0.1 ml of the dilution was plated on plate count agar (Oxoid, Italy) and incubated at 37 °C for 24–48 h to determine their concentration. For inoculation, portions of 600 g seeds were immersed in 800 ml of each appropriate inoculum suspension with gentle swirling for 5 min at room temperature. The final concentration of each inoculated microorganism in the seeds was approximately 2–3 log CFU/g. The seeds were air-dried overnight under a laminar flow biological safety hood at room temperature and stored at 4 ± 2 °C for up to 48 h. A part of the seeds were then subjected to sprouting, and a part of the seeds were directly treated with chlorine water to reduce the level of contamination.

2.5. Sprouting

The inoculated mung bean seeds (20 g) were placed in polypropylene sprouting jars and soaked in sterile distilled water for 24 h at room temperature (22 ± 1 °C). Each jar was then kept in the dark at 22 ± 1 °C with a relative humidity of 75 ± 5% for 5 days and rinsed with distilled water every 5 h daily. Fifteen sprouting jars for each microbial strain were used.

2.6. Mung bean sprout sampling

Three jars for each microbial strain were sampled as follows: a, seeds; b, seeds after soaking; c, 1st day of sprouting; d, 3rd day of sprouting and e, 5th day of sprouting. *L. monocytogenes* was counted by ISO 11290-2; *Salmonella* spp. was counted in brilliant green agar (Oxoid, Italy) according to ISO 6579-1 (2002) and STEC was counted in Sorbitol MacConkey agar SMAC (March and Ratnam, 1986). From each jar and at each time point, the seeds or sprouts were entirely collected, and 25 g were diluted and plated on the selective agars to quantify the inoculated pathogens. Briefly, *Listeria* selective agar, according to Otaviani and Agosti (ISO 11290-2), was used for *Listeria monocytogenes*. Then, twenty-five presumptive *L. monocytogenes* colonies were isolated for 5 randomly collected plates, and their identity was confirmed by RAPID[®] *Listeria*-mono from Bio-Rad (USA). Xylose Lysine Deoxycholate agar (XLD, Oxoid, Italy) was used for *Salmonella* spp. Then, 25 presumptive colonies were isolated from 5 randomly collected plates and streaked on brilliant green agar to confirm the presumptive identification. Finally, they were identified by API 20 E (bioMérieux, France).

Sorbitol McConkey agar (SMAC, March and Ratnam, 1986) was used to quantify STEC. Twenty five presumptive colonies were then isolated from 5 randomly collected plates and identified according to ISO CEN TS 13136 method.

2.7. Pathogen reduction by chlorinated water

Approximately 300 g of mung bean seeds were inoculated and sprouted, producing 15 samples of 20 g for each investigated microorganisms. After 5 days of sprouting, the samples were washed 4 times in chlorinated water (100 ppm) for 5 min each. After each chlorine treatment, the samples were floated for 5 min in acidulated water (10 ppm citric acid). Then, the sprouts were collected using a sterile spoon under a laminar flow hood and drained in a centrifuge (300 × g for 5 min). Three samples for each microorganism were analysed after each washing. Chlorine solution was prepared by adding 2.5% sodium hypochlorite to 0.05 M potassium phosphate buffer (pH 6.8, 21 °C) and then was diluted to reach the final concentration (100 ppm). The free chlorine in solution and the residual chlorine in the sprouts after each washing were determined with a chlorine test kit (Hach Lange GmbH, Germany). To determine the residual chlorine, sprouts were homogenized in distilled water (1/1) and centrifuged at 300 × g, and the supernatant was subjected to analysis by chlorine test kit (Hach Lange GmbH, Germany). The microbial reduction is shown as the concentration levels of surviving cells.

For the mung bean seeds, the part of the seeds initially inoculated for sprouting was treated with chlorinated water (100 ppm). In particular, 10 seed samples (10 g) of each strain were analysed after the inoculum (control), and 10 seed samples were analysed after chlorinated water treatment. Both the treated and untreated samples were analysed by the above mentioned method, and the treated samples were also analysed by enrichment methods as follows: ISO 11290-1 for *L. monocytogenes*; ISO 6579-1 for *Salmonella* spp.; and ISO TS 13136 for STEC. Data are given as the concentration of surviving cells. The free chlorine in solution and the residual chlorine in the seeds after washing were determined with a chlorine test kit (Hach Lange GmbH, Germany). To determine the residual chlorine, seeds were homogenized in distilled water (1/1) and centrifuged at 300 × g, and the supernatant was subjected to analysis by chlorine test kit (Hach Lange GmbH, Germany).

2.8. Statistical analysis

The values of the various parameters were compared by one-way analysis of variance. The averages of the microbial loads were compared with Tukey's honest significance test using StatGraphics software package from Statistical Graphics (Rockville, Maryland) ($p < 0.05$).

3. Results and discussion

The mean and the standard deviation of the TVC, total coliforms and *E. coli* in the mung bean seeds and sprouts were investigated over a period of 5 years from 2012 to 2016. In the seeds, the TVC means were less than 4.1 log CFU/g, which are quite similar to the means of the total coliforms, demonstrating that the coliforms are contaminants of mung bean seeds (Table 1). In the sprouts, the TVC concentration was less than or equal 5.5 log CFU/g, and the total coliform counts (TCCs) were less than or equal to 3.5 log CFU/g (Table 2). The *E. coli* concentration was consistently present at less than the lower detection limit of the method (< 10 CFU/g) in both mung bean seeds and sprouts, which also confirmed the total absence of STEC in 25 g. In addition, *Salmonella* spp. or *L. monocytogenes* were not detected either at the seed or sprout level. Both TVC and TCC loads did not have significant differences throughout the five years of investigation ($p > 0.05$).

Seeds contain a large microbial concentration ranging between 3 and 7 log CFU/g (Peles et al., 2012), and these levels can increase

during sprouting, reaching up to 11 log CFU/g (Peñas et al., 2008; Gabriel et al., 2007; Ghandi and Matthews, 2003; Lang et al., 2000; Prokopowich and Blank, 1991). Surveys have revealed that the total aerobic counts can reach levels of 3–6 log CFU/g for alfalfa seeds (Bayer et al., 2014; Prokopowich and Blank, 1991; Andrews et al., 1982, 1979), up to 4 log CFU/g for mung beans (Andrews et al., 1982), up to 5 log CFU/g for onion seeds (Prokopowich and Blank, 1991) and up to 7 log CFU/g for rice seeds (Piernas and Guiraud, 1997). In our investigation, the TVC seed contamination was lower than those obtained by the above authors.

The mung bean seed microorganisms originated from the soil and from the technological method of plant production. The investigated seeds originated from Australia, and both the technological production and the environmental parameters of the producing area are unknown. Considering the low level of contamination, it was concluded that the seeds probably come from an area where good production practices are strictly applied.

The TVC loads of the investigated sprouts produced and sold in Italy were also lower than those produced and sold in other parts of Europe and the world (Jeddi et al., 2014; Abadias et al., 2009). Several authors reported a TVC of between 8 and 11 log CFU/g in alfalfa, mung bean or onion sprouts Peñas et al., 2008; Gabriel et al. (2007); Ghandi and Matthews (2003); Viswanathan and Kaur (2001); Lang et al. (2000). Bacterial growth is favoured by the traditional sprouting conditions determined by 2–7 days of sprouting, with temperatures of 22–24 °C and physicochemical characteristics, such as optimum nutrient availability, pH and water activity (Peles et al., 2012; Taormina et al., 1999a). In Italy, the sprouting of mung bean seeds takes five days. Considering the means of the initial TVC and TCC and the means of both microbial groups in the sprouts at the moment of their harvesting, it appears that the increase in the TVC and TCC was approximately 1.8 and 0.4 log CFU/g, respectively. The increasing TVC levels were less than those observed by other authors. In particular, Peles et al. (2012) observed during sprout production that the TVC level continuously increased day after day up to 3 log CFU/g. In particular, it appeared that the increase was due to the kind of seeds, such as kidney beans, rice, wheat, lupin, fenugreek, alfalfa and mung beans (Weiss et al., 2007; Martínez-Villaluenga et al., 2006; Kimanya et al., 2003; Splittstoesser et al., 1983; Andrews et al., 1982).

The TCC in the investigated sprouts was also less than that observed in other studies, where a level of growth of up to 2–3 log CFU/g during sprouting was found. The level of increase of the TCC depended on the kinds of seeds (Peles et al., 2012; Weiss et al., 2007; Martínez-Villaluenga et al., 2006; Soylemez et al., 2001; Piernas and Guiraud, 1997; Prokopowich and Blank, 1991). For example, Peles et al. (2012) found that the TC increased for up to 3.5 log cycles CFU/g. The low TVC and TCC values in the investigated sprouts were due to the use of a sanitized apparatus and drinking water for watering. In each case, the final TVC value did not allow the shelf life to be prolonged for over 5 days for the investigated sprouts; even though TVC exceeded 7 log CFU/g, it did not negatively affect the sprout appearance (Taormina et al., 1999a).

Seeds may contain various microbial loads (Bayer et al., 2014; Peles et al., 2012), represented by environmental microorganisms, such as enterobacteria, *Pseudomonas*, lactic acid bacteria and yeast, which can rapidly grow (Randazzo et al., 2009; Robertson et al., 2002; Prokopowich and Blank, 1991) and reach high concentration levels (> 10 log CFU/g) (Peñas et al., 2008; Ghandi and Matthews, 2003; Prokopowich and Blank, 1991; Patterson and Woodburn, 1980). Our study focuses on the isolation and identification of coliforms, which represent the main microorganisms contaminating either seeds or sprouts. Table 3 reports the strains isolated from the VRBLA agar. As shown, the main strains were *Enterobacter cloacae* and *Klebsiella oxytoca*. *Klebsiella pneumoniae*, *K. aerogenes* and *Citrobacter freundii* were also isolated. Considering the isolated strains, it could be concluded that they originated from environmental contamination (Galli and Franzetti,

Table 1
Microbial quality of mung bean seeds.

Microbial group	Year of sampling				
	2012	2013	2014	2015	2016
Total viable count ^a	3.5 ± 0.1a	4.1 ± 0.5a	3.3 ± 0.3a	3.2 ± 0.8a	3.0 ± 1.0a
Total coliforms ^a	3.1 ± 0.2a	2.8 ± 0.4a	3.1 ± 0.1a	2.8 ± 0.4a	2.7 ± 0.4a
<i>Escherichia coli</i> ^b	< 10	< 10	< 10	< 10	< 10
<i>Salmonella</i> spp. ^c	Absence	Absence	Absence	Absence	Absence
<i>L.monocytogenes</i> ^c	Absence	Absence	Absence	Absence	Absence
STEC ^{c, d}	Absence	Absence	Absence	Absence	Absence

Legend.

^a log CFU/g.

^b CFU/g.

^c In 25 g.

^d Shiga Toxin *E. coli*. Mean with different letters within a row (following the lines) are significantly different ($p < 0.05$); 72 samples/year.

1991; Splittstoesser et al., 1983).

The packaging used (although there is an atmosphere consisting of air and a high relative humidity of 85–100%) and the storage temperature did not appear to favour the development of coliforms and other spoiling microorganisms.

Both TVC and TCC can grow in mung bean sprouts during 5 days of storage at $4 \pm 2^\circ\text{C}$ ($p < 0.05$). As shown in Table 4, in the investigated sprouts, both the TVC and the TCC increased up to 1 log CFU/g. Although Galli and Franzetti (1991) demonstrated that in fresh vegetable products, the predominant microorganisms grow, cause spoilage and depreciate vegetables, so they lose consistency and oxidize; it was observed that in mung bean sprouts, the bud that was initially ivory-white became orange/rust at the end of the shelf life as a consequence of the phenomena of oxidation and chemical and enzymatic browning (data not shown). It can thus be concluded that microorganisms appear to have only a marginal role in the decay of these products.

In the last decade, the consumption of sprouted seeds has increased in many countries, particularly in Italy (www.corriereortofrutticolo.it; www.bonduelle.it). However, this consumption has led to an increase in the number of sprout-associated food-borne illness outbreaks, with at least 40 outbreaks reported in several countries (Bayer et al., 2014; Health Canada, 2012; Fett, 2005), and the most frequently associated causative agents have been *Salmonella* and *E. coli* O157:H7 (CDC, 2012; Fett, 2005). Mung bean sprouts were responsible for over 600 reported cases of a salmonellosis outbreak in parts of Canada in 2005 (Health Canada, 2012). Similarly, in other vegetable sprouts, contamination by enterohemorrhagic *E. coli* was responsible for outbreaks in Europe (Bayer et al., 2014; Ding and Fu, 2016; Baranzoni et al., 2014). Consequently, mung bean sprouts constitute a significant food safety risk (FDA, 2016; EFSA, 2011a, 2011b, 2011c, 2012) because they are eaten raw in many countries, including Europe, the USA and Mexico. The

Table 2
Microbial quality of mung bean sprouts.

Microbial group	Year of sampling				
	2012	2013	2014	2015	2016
Total viable count ^a	5.5 ± 0.3a	5.2 ± 1.1a	5.3 ± 0.8a	5.1 ± 0.5a	5.1 ± 0.4a
Total coliforms ^a	3.4 ± 0.8a	3.3 ± 1.1a	3.2 ± 0.5a	3.5 ± 0.4a	3.1 ± 0.9a
<i>Escherichia coli</i> ^b	< 10	< 10	< 10	< 10	< 10
<i>Salmonella</i> spp. ^c	Absence	Absence	Absence	Absence	Absence
<i>L.monocytogenes</i> ^c	Absence	Absence	Absence	Absence	Absence
STEC ^{c, d}	Absence	Absence	Absence	Absence	Absence

Legend.

^a In log CFU/g.

^b In CFU/g.

^c In 25 g.

^d Shiga Toxin *E. coli*. Mean with different letters within a row (following the lines) are significantly different ($p < 0.05$); 72 samples/year.

Table 3
Identification of coliform strains isolated from sprouts.

Strains	%	Accession number
<i>Klebsiella pneumoniae</i>	14	NZ_CP006662.2
<i>Klebsiella oxytoca</i>	20	NZ_PCMV01000088.1
<i>Klebsiella (Enterobacter) aerogenes</i>	12	NZ_MTZP01001321.1
<i>Citrobacter freundii</i>	14	NZ_KQ464182.1
<i>Enterobacter cloacae</i>	40	NG_050406.1

Table 4
Fate of Total Viable Count of bacteria (TVC) and Total Coliforms counts (TCC) during the storage at $4 \pm 2^\circ\text{C}$ for 5 days of mung bean sprouts.

Days	TVC	TCC
	log CFU/g	
0	5.2 ± 0.1a	3.2 ± 0.1a
5	6.8 ± 0.4b	4.5 ± 0.2b

Legend: Mean with different letters within a row (following the columns) are significantly different ($p < 0.05$).

absence of any heat treatment increases the potential infection risk associated with sprout consumption (Knödler et al., 2016; Sadler-Reeves et al., 2015), as bacterial pathogens can contaminate fresh vegetables as primary or secondary contamination (Knödler et al., 2016; Harris et al., 2003). However, sprout-associated outbreaks have largely been linked to seeds contaminated with pathogenic microorganisms (Knödler et al., 2016; NACMCF, 1999) rather than post-production contamination. EFSA (2012) reported over 50 outbreaks, with *Salmonella* and STEC being the most frequently responsible agents. The largest outbreak occurred in 2011 in Germany due to Shiga toxin-producing *E. coli* O104:H4 present in fenugreek sprouts grown on an organic

farm and obtained from contaminated seeds (Buchholz, 2011), which were imported from Egypt.

Pathogens usually contaminate seeds (Beales, 2004; NACMCF, 1999; Mahon et al., 1997). Consequently, seeds are generally recognized as the main source of bacterial pathogens in most sprout-related outbreaks reported by the NACMCF, 1999 and various authors (Peles et al., 2012; Taormina et al., 1999). The control of each lot of seeds used to produce the investigated sprouts did not demonstrate the presence of either STEC, *Salmonella* spp. or *L. monocytogenes*. According to the Commission Regulation (EU) No. 209/2013, Italian producers control the seeds and the sprouts before the sale for the presence of STEC, which has never been found in any of the lots of mung bean seeds or in the sprouts made with those seeds. Again, the main producer in Italy has demonstrated the absence of the above three species of pathogens since 1982, when they began production. Since 1982, sprouts from this producer have been sampled monthly by Italian Official Control Laboratories (ATS-Azienda Sanitaria Territoriale) to monitor the presence of pathogenic microorganisms, and they have never found STEC, *L. monocytogenes* or *Salmonella* spp. (Producer, personal communication).

Considering the potential outbreak problems in Europe and in many other parts of the world, and despite the total absence of pathogenic microorganisms in the investigated sprouts, the additional aim was to study the fate of pathogenic microorganisms during mung bean seed sprouting and a method based on chlorinated water to decontaminate them before selling. As expected, all the investigated pathogenic strains grew on sprouts, reaching levels of approximately 9 log CFU/g within 5 days of sprouting (Table 5). After one day of sprouting, the level of the inoculated strains was similar to the initial inocula. At 3 days of sprouting, the strains grew up to 7–8 log CFU/g ($p < 0.05$), and at 5 days of sprouting, the counts reached values up to approx. 9 log CFU/g ($p < 0.05$). The watering, the water and the sprouting room temperatures permit the growth of microorganisms. The temperature affects the growth of *E. coli* and other pathogenic microorganisms, as demonstrated by Gómez-Aldapa et al. (2015) in mung bean sprouts and by Charkowski et al. (2002) in alfalfa seeds. Gómez-Aldapa et al. (2015) demonstrated that all *E. coli* pathotype (DEP) diarrhoeagenic strains grew in mung bean seeds sprouted at $20 \pm 2^\circ\text{C}$ or $30 \pm 2^\circ\text{C}$, reaching 5 log and 7 log CFU/g, respectively.

Our data are in agreement with the previous research of Warriner et al. (2003) and Castro-Rosas and Escartín (2000), who observed up to 5 log CFU/g of *Salmonella* Typhi and *E. coli* O157:H7 growth during the processes of germinating and sprouting of alfalfa seeds and mung bean seeds, respectively. Again, Xiao et al. (2014) observed high levels of survival and growth of *E. coli* O157:H7 and O104:H4 during radish sprouting and microgreens, which were responsible for recent food-borne cases in Germany.

Salmonella and *L. monocytogenes* also represent a microbial risk for mung bean sprouts and other sprouted vegetables. The EFSA (2011a,b,c) reported that 34 out of 43 outbreaks were associated with the consumption of various sprouted seeds contaminated by *Salmonella*.

L. monocytogenes and *Salmonella* spp. can grow, as demonstrated in this work, during mung bean sprouting (Table 5). Piernas and Guiraud (1997) found that *L. monocytogenes*, inoculated in rice seeds, grew

during germination without any apparent antagonist from the background microbiota. *L. monocytogenes* appears to be rarely present in seeds and sprouts (Symes et al., 2015; Palmai and Buchanan, 2002), but it has been implicated in some incidents (FDA, 2016; CDC, 2014).

In terms of the risk for consumers, different treatments have been tested to reduce seed contamination by food pathogens (Nei et al., 2013; Studer et al., 2013; Fransisca et al., 2012; Kim et al., 2010; Taormina and Beuchat, 1999). Physical (e.g., dry heat, hot water, high hydrostatic pressure, and irradiation), biological (e.g., antagonistic microorganisms and their metabolites) and chemical process (i.e., chlorine, ozone and organic acids) strategies (Ding and Fu, 2016; Sikin et al., 2013) have been explored to minimize the risks associated with bacterial pathogens on seeds and beans used for sprouting (Trzaskowska et al., 2018). Disinfecting seeds and sprouts remains the most important step in the safety of vegetable sprouts (Trzaskowska et al., 2018). The Canadian Food Inspection Agency (CFIA, 2007) and the FDA (2016, 2014) recommend a minimum of 3 log of microbial reduction in seeds by the application of a sanitation step.

In this work, the use of chlorinated water to reduce sprout contamination was investigated. As shown in Table 6, it was suggested that mung bean sprouts can be decontaminated through 4 washings with chlorinated water. The initial contamination was up to 9 log CFU/g and represents the concentration of the inoculated seeds after sprouting. The 4 washings with chlorinated water (100 ppm) decreased the contamination to less than 2 log CFU/g, a decrease higher than that suggested by both the CFIA (2007) and the FDA (2016, 2014). However, this treatment led to three problems. First, the use of a chlorine solution to wash sprouts is illegal in Italy, but it could be acceptable if no chlorine residues are present in sprouts; second, residual chlorine could remain in the sprouts; and third, the chlorine rapidly changes the colour of the sprouts to pale yellow or orange-rust. After traditional washing with a solution of citric acid, the colour of the sprouts is white, which is widely accepted by consumers. In contrast, consumers consider that a pale-yellow colour as an oxidative index. In addition, after opening the treated sprout packaging, an intense chlorine smell was produced, despite additional washing with drinking water after treatment. The treatment with 100 ppm chlorine reduced the pathogens up to 9 log CFU/g, but after washing, chlorine residues of 10–15 ppm remained. Thus, the method cannot be used in Italy. It was not possible to reduce the chlorine concentration to 20–30 ppm for the treatment because this concentration is not sufficient to reduce the contamination of the investigated pathogenic microorganisms up to 3 log CFU/g (data not shown). Thus, it can be concluded that the seeds must be directly decontaminated before sprouting, as suggested by other authors (Trzaskowska et al., 2018; Knödler et al., 2016). Our data (Table 7) demonstrate that it is possible to reduce the initial contamination by approximately 3 log CFU/g, even though the treatment did not ensure zero tolerance for the inoculated pathogenic strains. It was impossible to completely eradicate the pathogens. At the end of the seed chlorine treatment, the pathogens were detected only by enrichment (Table 7), and 20 ppm residual chlorine in sprouts was found. In each case, the use of chlorine at high or low concentrations either in sprouts or in seeds does not completely eliminate the hazard or the risk of the presence of pathogenic microorganisms. Other authors used combined treatments

Table 5

Fate of different pathogenic microorganisms intentionally inoculated during mung beans sprouting.

Microorganisms	Seed	Seed after soaking	After 1st day of sprouting	After 3rd day of sprouting	After 5th day of sprouting
<i>Salmonella</i> spp.	2.5 ± 0.2a	2.7 ± 0.1a	2.7 ± 0.3a	8.1 ± 0.1b	9.1 ± 0.8b
<i>L. monocytogenes</i>	2.1 ± 0.2a	2.3 ± 0.2a	2.5 ± 0.3a	7.2 ± 0.1b	9.6 ± 0.3c
STEC ^a	2.1 ± 0.3a	2.2 ± 0.1a	2.4 ± 0.2a	8.5 ± 0.4b	9.8 ± 0.1c

Legend.

^a Shiga Toxin *E. coli*; data in log CFU/g mean ± standard deviation of 3 repetitions/condition; Mean with different letters within a row (following the lines) are significantly different ($p < 0.05$).

Table 6

Surviving cells concentration of the investigated pathogens in sprouts after 4 washings in chlorinated water solution (100 ppm).

Microorganisms	Before washing	After 1st Washing	After 2nd washing	After 3rd washing	After 4th washing
<i>Salmonella</i> spp.	9.1 ± 0.8a	7.7 ± 0.3b	5.4 ± 0.3c	2.1 ± 0.1d	1.8 ± 0.2d
<i>L. monocytogenes</i>	9.6 ± 0.3a	7.3 ± 0.4b	5.5 ± 0.3c	2.2 ± 0.2d	1.9 ± 0.2d
STEC ^a	9.8 ± 0.1a	7.2 ± 0.2b	5.4 ± 0.2c	1.9 ± 0.4d	1.8 ± 0.1d

Legend.

^a Shiga Toxin *E. coli*; data in log CFU/g mean ± standard deviation of 3 repetitions/condition; Mean with different letters within a row (following the lines) are significantly different ($p < 0.05$). Chlorine residual in sprouts after 4 washing: 10–15 ppm.

Table 7

Surviving cells concentration of the investigated pathogens in seeds after chlorine treatment.

Microorganisms	Seeds log CFU/g	After soaking CFU/g	After soaking Presence in 25 g
<i>Salmonella</i> spp.	3.1 ± 0.2	< 10	+
<i>L. monocytogenes</i>	3.6 ± 0.1	< 10	+
STEC ^a	3.3 ± 0.2	< 10	+

^a Shiga Toxin *E. coli*; seeds treated in chlorinated water solution (100 ppm); Chlorine residual in seeds after 4 washing: 20 ppm; Time of soaking: 60 min.

or hurdles to reduce the level of undesirable microorganisms on seeds and considered them more effective than individual treatments (Ding et al., 2013; Nei et al., 2013). Most recently, both *S. enterica* and *E. coli* O157:H7 were reduced by up to 5 log CFU/g on mung beans washed with hot water at 85 °C for 40 s, followed by soaking in a 2000 ppm chlorine solution for 2 h. However, the authors did not report the value of residual chlorine or the presence of the chlorine smell in the sprouts, as demonstrated in our work using a 100 ppm chlorine solution. In addition, other methods, including ozonated water (Sharma et al., 2002), pulsed ultraviolet light (Sharma and Demirci, 2003), γ -radiation (Thayer et al., 2003), single or combined treatment with hot water followed by exposure to H₂O₂ (Hong and Kang, 2016) or to H₂O₂ and acetic acid (Trzaskowska et al., 2018; Studer et al., 2013) or only with acetic acid (Lang et al., 2000) and ultrasound followed by a washing solution of ClO₂ (3 ppm) (Millan-Sango et al., 2017; Holliday et al., 2001; Beuchat et al., 1997), have been evaluated to decontaminate vegetable sprouts. Both single and combined treatments had a good bactericidal effect (up to 3–5 log CFU/g) and significant reduction impacts ($p < 0.05$), but they did not completely eliminate *E. coli* and *Salmonella* on alfalfa and mung bean sprouts. Thus, there is no guarantee of seed treatment that is able to eradicate contamination from bacterial pathogens before seed germination (Knödler et al., 2016). With respect to avoiding sprout-related STEC, *L. monocytogenes* and *Salmonella* spp. outbreaks in the future, it is suggested that a short treatment of chlorinated water washing for the seeds can be effective, as tested using single or combined procedures (Knödler et al., 2016) that are cheap and already included in the FDA guidelines for sprout production in the US (Knödler et al., 2016; HPA, 2009; NACMCF, 1999).

4. Conclusion

This study provides information on the hygienic quality and microbial status of mung bean sprouts produced in Italy. The microbial loads were less than those observed in seeds and sprouts produced in other countries, and in particular, the pathogenic microorganisms responsible for recent outbreaks (Buchholz, 2011; Health Canada, 2012; EFSA, 2012) were not present.

However, it was confirmed that pathogenic microorganisms can grow during mung bean sprout production. Consequently, single or combined procedures of decontamination must be applied. Decontamination using a chlorine solution seems to be useful, but

various chlorine residues remained in sprouts. Despite these encouraging results, attention to the hygienic quality should not be reduced, and it is of great importance to minimize bacterial contamination. In particular, it is suggested that pathogenic microorganisms are reduced and eliminated directly from seeds, and thus the microbial contamination of sprouts can be minimized before consumption. Despite the time spent soaking seeds (60 min) or washing sprouts (4 washings with chlorine followed by acidulated water, each of them for 5 min), the suggested chlorine method might be feasible for industrial purposes because it permits the reduction of contamination.

References

- Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I., 2009. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *Int. J. Food Microbiol.* 123, 121–129.
- Altschul, S.F., Madden, T.L., Shaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402.
- Andrews, W.H., Wilson, C.R., Poelma, P.L., Romero, A., Mislivec, P.B., 1979. Bacteriological survey of sixty health foods. *Appl. Environ. Microbiol.* 37, 559–566.
- Andrews, W.H., Mislivec, P.B., Wilson, C.R., Bruce, V.R., Polema, P.L., Gibson, R., Trucksess, M.W., Young, K., 1982. Microbial hazards associated with bean sprouting. *JAOC (J. Assoc. Off. Anal. Chem.)* 65, 241–248.
- FDA, 2014. FDA Investigates Multistate Outbreak of *Salmonella* Enteritidis Linked to Mung Bean Sprouts Produced by Wonton Foods Inc. Of New York. (Available at: <http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm424426.htm>).
- Baranzoni, G.M., Fratamico, P.M., Rubio, F., Glaze, T., Bagi, L.K., Albonetti, S., 2014. Detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC) O104 from sprouts. *Int. J. Food Microbiol.* 173, 99–104.
- Bayer, C., Bernard, H., Prager, R., Rabsch, W., Hiller, P., Malorny, B., Pfefferkorn, B., Frank, C., de Jong, A., Friesema, I., Stark, K., Rosner, B.M., 2014. An outbreak of *Salmonella* Newport associated with mung bean sprouts in Germany and The Netherlands, October to November 2011. *Euro Surveill.*, vol. 19 (1) (pii = 20665). Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20665>.
- Beales, N., 2004. Review No. 41. Review of the microbiological risk associated with sprouted seeds. Campden and Chorleywood Food Research Association Group (72 pp.).
- Beuchat, L.R., 1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. *Int. J. Food Microbiol.* 34, 329–333.
- Buchholz, U., 2011. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *N. Engl. J. Med.* 365, 1763–1770.
- Castro-Rosas, J., Escartin, E.F., 2000. Survival and growth of *Vibrio cholerae* O1, *Salmonella typhi* and *Escherichia coli* O157:H7 in alfalfa sprouts. *J. Food Sci.* 65, 162–165.
- CDC, 2012. *Escherichia coli*. Available: www.cdc.gov/ecoli/general/#who, Accessed date: 20 March 2018.
- CDC, Centers for Disease Control and Prevention, 2014. Wholesome soy products, inc. Sprouts and Investigation of Human Listeriosis Cases accessed 6.16.16. <http://www.cdc.gov/listeria/outbreaks/bean-sprouts-11-14/index.html>.
- CFIA, Canadian Food Inspection Agency, 2007. Code of practice for the hygienic production of sprouted seeds accessed 9.6.16. <http://www.inspection.gc.ca/food/fresh-fruits-and-vegetables/food-safety/sprouted-seeds/eng/1413825271044/1413825272091#a8>.
- Charkowski, A.O., Barak, J.D., Sarreal, C.Z., Mandrell, R.E., 2002. Differences in growth of *Salmonella enterica* and *Escherichia coli* O157:H7 on alfalfa sprouts. *Appl. Environ. Microbiol.* 68, 3114–3120.
- Cleary, P., Browning, L., Coia, J., Cowden, J., Fox, A., Kearney, J., Lane, C., Mather, H., et al., 2010. A foodborne outbreak of *Salmonella* Bareilly in the United Kingdom. *Euro Surveill.* 15, 1–3.
- Cocolin, L., Astori, G., Manzano, M., Cantoni, C., Comi, G., 2000. Development and evaluation of a PCR-microplate capture hybridization method for direct detection of verotoxigenic *E. coli* strains in artificially contaminated food samples. *Int. J. Food Microbiol.* 54, 1–8.
- Commission regulation (EU) No 209/2013 of 11 March 2013 amending Regulation (EC) No 2073/2005 as regards microbiological criteria for sprouts and the sampling rules for poultry carcasses and fresh poultry meat. *Off. J. Eur. Union.* L68/19.
- Ding, H., Fu, T.J., 2016. Assessing the public health impact and effectiveness of interventions to prevent *Salmonella* contamination of sprouts. *J. Food Prot.* 79, 37–42.

- Ding, H., Fu, T.J., Smith, M.A., 2013. Microbial contamination in sprouts: how effective is seed disinfection treatment? *J. Food Sci.* 78, R495–R501.
- EFSA, 2011. Scientific opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA J* 9, 2424.
- EFSA, 2011. Tracing Seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in relation to the shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Germany and France. 2011.
- EFSA, 2011. Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. *EFSA J* 9, 2274 *Euro Surveill.* 19 (1) (pii = 20665. Available at: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20665>.
- EFSA, 2012. Scientific opinion on the risk posed by Shiga toxin-producing *Escherichia coli* (STEC) and other pathogenic bacteria in seeds and sprouted seeds. *EFSA J* 9, 1–101.
- FDA, US Food and Drug Administration, 2016. Outbreaks - outbreak investigations accessed 6.16.16. <http://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm272351.htm>.
- Fett, W.F., 2005. Interventions to ensure the microbiological safety of sprouts. In: Sapers, G.M., Gorny, J.R., Yousef, A.E. (Eds.), *Microbiology of Fruit and Vegetables*. CRC Press, Boca Raton, FL, pp. 187–210.
- Francisca, L., Park, H.K., Feng, H., 2012. *E. coli* O157:H7 population reduction from alfalfa seeds with malic acid and thiamine dilauryl sulfate and quality evaluation of the resulting sprouts. *J. Food Sci.* 77, M121–M126.
- Gabriel, A.A., Berja, M.C., Estrada, A.P., Lopez, M.A., Nery, J.B., Villafior, E.B., 2007. Microbiology of retail mung bean sprouts vended in public markets of National Capital Region, Philippines. *Food Control* 18, 1307–1313.
- Galli, A., Franzetti, L., 1991. I batteri Gram negativi nelle insalate della IV gamma. *Ann. Microbiol.* 41, 41–51.
- Ghandi, M., Matthews, K.R., 2003. Efficacy of chlorine and calcinated calcium treatment of alfalfa seeds and sprouts to eliminate Salmonella. *Int. J. Food Microbiol.* 87, 301–306.
- Gómez-Aldapa, C.A., Rangel-Vargas, E., Bautista-De León, H., Vázquez-Barrios, M.E., Gordillo-Martínez, A.J., Castro-Rosas, J., 2015. Behavior of enteroaggregative *Escherichia coli*, non-O157-shiga toxin-producing *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli* and enterotoxigenic *E. coli* strains on mung bean seeds and sprout. *Int. J. Food Microbiol.* 166, 364–368.
- Harris, L.J., Farber, J.N., Beuchat, L.R., Parish, M.E., Suslow, T.V., Garrett, E.H., Busta, F.F., 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh cut produce. *Compr. Rev. Food Sci. F.* 2, 78–141.
- Health Canada, 2012. Risk associated with sprouts. Available at: Accessed. <http://www.hc-sc.gc.ca/hl-vs/iyh-vsv/food-aliment/sprouts-germes-eng.php>, Accessed date: 31 July 2013.
- Health Protection Agency, 2009. Guidelines for Assessing the Microbiological Safety of Ready-To-Eat Foods Placed on the Market. HPA, London.
- Holliday, S.L., Scouten, A.J., Beuchat, L.R., 2001. Efficacy of chemical treatments in eliminating *Salmonella* and *Escherichia coli* O157:H7 on scarified and polished alfalfa seeds. *J. Food Prot.* 64, 1489–1495.
- Hong, E.J., Kang, D.H., 2016. Effect of sequential dry heat and hydrogen peroxide treatment on Reduction of *Salmonella Typhimurium* on alfalfa seeds and seeds germination. *Food Microbiol.* 53, 9–14 Part B.
- Iacumin, L., Cecchini, F., Manzano, M., Osualdini, M., Boscolo, D., Orlic, S., Comi, G., 2009. Description of the microflora of sourdoughs by culture-dependent and culture independent methods. *Food Microbiol.* 26, 128–135.
- ISO 11290-1,2:1996 Adm.1, 2004. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Listeria Monocytogenes*) Method.
- ISO 4832, 2006. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Coliforms – Colony-count Technique.
- ISO 6579-1, 2002. Cor.1:2004 Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Salmonella* Spp. Method.
- ISO CEN TS 13136 - Commission Regulation EU N. 209/13 - 11/03/2013 Amending Regulation EC 2073/2005 as Regards Microbiological Criteria for Sprouts and the Sampling Rules for Poultry Carcasses and Fresh Poultry Meat (Official J. European Union L 68/19, 12/01/2013) method.
- Jeddi, M.Z., Yunesian, M., Gorji, M.E., Noori, N., Pourmand, M.R., Khaniki, G.R.J.K., 2014. Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *J. Health Popul. Nutr.* 32, 391–399.
- Kim, H., Bang, J., Beuchat, L.R., Ryu, J.H., 2010. Synergistic effect of chlorine dioxide and drying treatments for inactivating *Escherichia coli* O157:H7 on radish seeds. *J. Food Prot.* 73, 1225–1230.
- Kimanya, M.E., Mamiro, P.R.S., Van Camp, J., Devlieghere, F., Opsomer, A., Kolsteren, P., Debevere, J., 2003. Growth of *Staphylococcus aureus* and *Bacillus cereus* during germination and drying of finger millet and kidney beans. *Int. J. Food Sci. Technol.* 38, 119–125.
- Klijn, N., Weerkamp, A.H., de Vos, W.M., 1991. Identification of mesophilic lactic acid bacteria by using polymerase chain reaction-amplified variable regions of 16S rRNA and specific DNA probes. *Appl. Environ. Microbiol.* 57, 3390–3393.
- Knödler, M., Berger, M., Dobrindt, U., 2016. Long-term survival of the Shiga toxin-producing *Escherichia coli* O104:H4 outbreak strain on fenugreek seeds. *Food Microbiol.* 59, 190–195.
- Kuo, Y.H., Rozan, P., Lambein, F., Frias, J., Vidal-Valverde, C., 2004. Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. *Food Chem.* 86, 537–545.
- Lang, M.M., Ingham, B.H., Ingham, S.C., 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* O157:H7 from alfalfa seeds prior to sprouting. *Int. J. Food Microbiol.* 58, 73–82.
- Mahon, B.E., Ponka, A., Hall, W., Komatsu, K., Beuchat, L., Shiflett, S., Siitonen, A., Cage, G., Lambert, M., Hayes, P., Bean, N., Griffin, P., Slutsker, L., 1997. An international outbreak of *Salmonella* infections caused by alfalfa sprouts grown from contaminated seed. *J. Infect. Dis.* 175, 876–882.
- March, S.B., Ratnam, S., 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.* 23, 869–872.
- Martínez-Villaluenga, C., Gulewicz, P., Pérez, A., Frías, J., Vidal-Valverde, C., 2006. Influence of lupin (*Lupinus luteus* L. cv. 4492 and *Lupinus angustifolius* L. var. Zapaton) and fenugreek (*Trigonella foenum-graecum* L.) germination on microbial population and biogenic amines. *J. Agric. Food Chem.* 54, 7391–7398.
- Martínez-Villaluenga, C., Frías, J., Gulewicz, P., Gulewicz, K., Vidal-Valverde, C., 2008. Food safety evaluation of broccoli and radish sprouts. *Food Chem. Toxicol.* 46, 1635–1644.
- Millan-Sango, D., Sammut, E., Van Impe, J.F., Valdramidis, V.P., 2017. Decontamination of alfalfa and mung bean sprouts by ultrasound and aqueous chlorine dioxide. *LWT, Food Sci. Technol.* 78, 90–96.
- NACMCF (National Advisory Committee on Microbiological Criteria for Food), 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *Int. J. Food Microbiol.* 52, 123–153.
- Nei, D., Bari, M.L., Enomoto, K., 2013. Validation of hot water and chlorine treatments to inactivate pathogens inoculated on mung bean seeds: influence of the seed production area. *Food Control* 32, 186–189.
- Palmari, M., Buchanan, 2002. Growth of *L. monocytogenes* during alfalfa sprouts. *Food Microbiol.* 19, 195–200.
- Patterson, J.E., Woodburn, M.J., 1980. *Klebsiella* and other bacteria on alfalfa and bean sprouts at the retail level. *J. Food Sci.* 45, 492–495.
- Peles, F., Györi, Z., Bácskai, T., Szabó, Zs, Murvai, M., Kovács, B., 2012. Microbiological quality of organic wheat grains and sprout. *Analele Universității din Oradea, Fascicula Protecția Mediului XVIII*, 53–60.
- Peñas, E., Gómez, R., Frías, J., Vidal-Valverde, C., 2008. Application of high pressure treatment on alfalfa (*Medicago sativa*) and mung bean (*Vigna radiata*) seeds to enhance the microbial safety of their sprouts. *Food Control* 19, 698–705.
- Piernas, V., Guiraud, J.P., 1997. Disinfection of rice seeds prior to sprouting. *J. Food Sci.* 62, 611–615.
- Prokopowich, D., Blank, G., 1991. Microbiological evaluation of vegetable sprouts and seeds. *J. Food Prot.* 54, 560–562.
- Randazzo, C.L., Scifo, G.O., Tomaselli, F., Caggia, C., 2009. Polyphasic characterization of bacterial community in fresh cut salads. *Int. J. Food Microbiol.* 128, 484–490.
- Robertson, L.J., Johannessen, G.S., Gjerde, B.K., Loncarevic, S., 2002. Microbiological analysis of seed sprouts in Norway. *Int. J. Food Microbiol.* 75, 119–126.
- Sadler-Reeves, L., Jorgensen, F., Lane, C., Willis, C., McLaughlin, J., Aird, H., de Pinna, E., Elviss, N., Fox, A., Kaye, M., 2015. The occurrence of *Salmonella* in raw and ready-to-eat bean sprouts and sprouted seeds on retail sale in England and Northern Ireland. *Lett. Appl. Microbiol.* 62, 126–129.
- Sharma, R.R., Demirci, A., 2003. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with pulsed ultraviolet light and response surface modeling. *J. Food Sci.* 68 (4), 1448–1453.
- Sharma, R.R., Demirci, A., Beuchat, L.R., Fett, W.F., 2002. Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with ozonated water and heat treatment. *J. Food Prot.* 65 (3), 447–451.
- Sikin, A.M.D., Zoellner, C., Rizvi, S.S.H., 2013. Current intervention strategies for the microbial safety of sprouts. *J. Food Prot.* 76 2099-2099.
- Soylemez, G., Brashears, M.M., Smith, D.A., Cuppett, S.L., 2001. Microbial quality of alfalfa seeds and sprouts after a chlorine treatment and packaging modifications. *J. Food Sci.* 66, 153–157.
- Spittstoesser, D.F., Queale, D.T., Andaloro, B.W., 1983. The microbiology of vegetable sprouts during commercial production. *J. Food Saf.* 5, 79–86.
- Studer, P., Heller, W.E., Hummerjohann, J., Drissner, D., 2013. Evaluation of aerated steam treatment of alfalfa and mung bean seeds to eliminate high levels of *Escherichia coli* O157:H7 and O178:H12, *Salmonella enterica*, and *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 79, 4613–4619.
- Symes, S., Goldsmith, P., Haines, H., 2015. Microbiological safety and food handling practices of seed sprout products in the Australian State of Victoria. *J. Food Prot.* 78, 1387–1391.
- Taormina, P.J., Beuchat, L.R., 1999. Comparison of chemical treatments to eliminate enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. *J. Food Prot.* 62, 318–324.
- Taormina, P.J., Beuchat, L.R., Slutsker, L., 1999. Infections associated with eating seed sprouts: an international concern. *Emerg. Infect. Dis.* 5, 626–634.
- Thayer, D.W., Boyd, G., Fett, W.F., 2003. γ -Radiation decontamination of alfalfa seeds naturally contaminated with *Salmonella mbandaka*. *J. Food Sci.* 68 (5), 1777–1781.
- Trzaskowska, M., Dai, Y., Delaquis, P., Wang, S., 2018. Pathogen reduction on mung bean reduction of *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* on mung bean using combined thermal and chemical treatments with acetic acid and hydrogen peroxide. *Food Microbiol.* 76, 62–68.
- Viswanathan, P., Kaur, R., 2001. Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. *Int. J. Hyg Environ. Health* 203, 205–213.
- Warriner, K., Spaniolas, S., Dickinson, M., Wright, C., Waites, W.M., 2003. Internalization of bioluminescent *Escherichia coli* and *Salmonella Montevideo* in growing bean sprouts. *J. Appl. Microbiol.* 95, 719–727.
- Weiss, A., Hertel, C., Grothe, S., Ha, D., Hammes, W.P., 2007. Characterization of the cultivable microbiota of sprouts and their potential for application as protective cultures. *Syst. Appl. Microbiol.* 30, 483–493.
- Xiao, Z., Nou, X., Luo, Y., Wang, Q., 2014. Comparison of the growth of *Escherichia coli* O157: H7 and O104: H4 during sprouting and microgreen production from contaminated radish seeds. *Food Microbiol.* 44, 60–63.
- Zheng, Q., Mikšs-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 Microbiol.* 52, 159–168.
- Zheng, Q., Mikšs-Krajnik, M., D'Souza, C., Yang, Y., Heo, D.J., Kim, S.K., Lee, S.C., Yuk, H.G., 2016. Evaluation of real-time PCR coupled with immunomagnetic separation or centrifugation for the detection of healthy and sanitizer-injured *Salmonella* spp. on mung bean sprouts. *Int. J. Food Microbiol.* 222, 48–55.