Innovative Food Science and Emerging Technologies 47 (2018) 180–186

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Inactivation of *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 inoculated on coriander by freeze-drying and supercritical CO_2 drying

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ABSTRACT

Coriander, either fresh or inoculated with three strains of *Escherichia coli* O157:H7, *Salmonella* or *Listeria monocytogenes*, was treated with supercritical CO_2 ($scCO_2$, with and without drying) or freeze-dried. After drying in $scCO_2$ for 150 min at 80 bar and 35 °C, the aerobic plate count, yeasts and molds, and the Enterobacteriaceae were reduced by 2.80, 5.03, and 4.61 log CFU/g, respectively. The total count of mesophilic aerobic spores was not significantly reduced by the treatment. Freeze-drying induced lower reductions with 1.23, 0.87, and 0.97 log CFU/g, respectively. After treatment at 100 bar and 40 °C without drying, inoculated strains of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were inactivated by > 7.37, > 4.73 and 4.99 log CFU/g, respectively. After drying in $scCO_2$ for 150 min at 80 bar and 35 °C, the strains were reduced by > 5.18 log CFU/g. Freeze-drying resulted in lower reduction with maximum 1.53, 2.03, and 0.71 log CFU/g, respectively. This study indicated that $scCO_2$ can be used for drying while offering a good inactivation of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* as well as most of the bacteria in the vegetative form naturally occurring on coriander.

Industrial relevance: Although dried foods are considered microbiological stable foods and show adverse conditions to microbial growth, they may still host pathogenic microorganisms, which may proliferate upon sufficient rehydration. Highly contaminated commodities such as herbs and spices can pose a threat to consumer health if not processed carefully. There is therefore a need to develop or improve drying techniques which can provide dried foods while reducing the initial contamination to acceptable levels in a single process. CO_2 is a cheap, accessible solvent, with a low critical point (31 °C, 73.8 bar). Moreover, in the supercritical region, CO_2 exhibits potent microbicidal properties. Therefore, supercritical CO_2 drying could be a valuable alternative non-thermal technique for conventional drying methods, such as air-drying or freeze-drying, when medium to high value-added food products with high initial contamination are involved.

1. Introduction

Dried products are increasingly consumed throughout the world and they are generally considered as safe. Unfortunately, although growth of spoilage and pathogenic microorganisms are inhibited in low water activity products, vegetative cells and spores can remain viable for a long time (Beuchat et al., 2013). In particular, *Salmonella* is known to be a resistant microorganism in a desiccated state (Finn, Condell, McClure, Amézquita, & Fanning, 2013) and has been the cause of a number of outbreaks and recalls in dried spices and herbs along with other hazards, such as *Bacillus cereus*, *Clostridium perfringens*, and Hepatitis A virus (Bourdoux, Li, Rajkovic, Devlieghere, & Uyttendaele, 2016).

Nowadays, most of the drying industry consists of sun-drying, convective air-drying, and freeze-drying. Sun-drying is indisputably the cheapest drying technique available but it is time-consuming and can lead to unsafe products due to microbial contamination before, during, and after drying (Karabulut, Topcu, Duran, Turan, & Ozturk, 2007). Air-drying techniques are faster but most of them are to be avoided for thermo-sensitive products such as fruits, vegetables, and herbs. For thermo-sensitive products, freeze-drying and other technologies

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https://doi.org/10.1016/j.ifset.2018.02.007
Received 11 September 2017; Received in revised form 22 January 2018; Accepted 7 February 2018
Available online 09 February 2018

Table 1
E. coli O157:H7, *L. monocytogenes*, and *Salmonella* strains with their respective selective medium, antibiotic resistance, and origin.

Strain	Selective medium	Antibiotic resistance	Origin
Escherichia coli O157:H7			
NCTC12900	Cefixime-Tellurite Sorbitol MacConkey Agar	Nalidixic acid (50 μg/mL)	Isolate from humans
BRMSID 188		Kanamycin (100 μg/mL)	Isolate from bovine
LFMFP 846		Nalidixic acid (50 μg/mL)	Isolate from beef carpaccio
Salmonella			
S. Thompson RM1987	Xylose Lysine Desoxycholate	Nalidixic acid (50 μg/mL)	Fresh coriander (cilantro)
S. Typhimurium SL 1344		Streptomycin (100 μg/mL)	Burke, Brözel, & Venter, 2008
S. Typhimurium LFMFP 883		Kanamycin (100 μg/mL)	Environmental isolate
Listeria monocytogenes			
LMG 23192	Agar Listeria Ottavani & Agosti	NA	Liver paste
LMG 23194		NA	Wijnendaele cheese
LMG 26484		NA	Isolate from tuna salad

involving a vacuum are often preferred as they are often considered the best techniques to preserve the organoleptic properties of the foodstuffs (Ratti, 2001). The main drawbacks of freeze-drying and low-pressure drying are the considerable costs and a low production rate (Yaghmaee & Durance, 2007). Microwave-assisted freeze-drying has been implemented to reduce the long drying time but many technical problems subsist such as corona discharges, overheating, and melting etc. (Ratti, 2001).

To overcome these disadvantages and practical issues, other adaptations and emerging technologies, such as supercritical CO_2 ($scCO_2$) drying, have been proposed for medium to high value-added product (Sagar & Suresh Kumar, 2010). $ScCO_2$ drying consists of the extraction of water from the product using $scCO_2$. Since water is not removed by vaporization or sublimation but is dissolved in the $scCO_2$, there are no thermal effects on the product quality (Benali & Boumghar, 2014). Until now, few studies have discussed $scCO_2$ drying of foods (Braeuer et al., 2017; Brown, Fryer, Norton, Bakalis, & Bridson, 2008; Khalloufi, Almeida-Rivera, & Bongers, 2010). However, drying of gels and preparation of catalysts with $scCO_2$ have been increasingly studied for the past 20 years (Brown, Fryer, Norton, & Bridson, 2010; García-González, Camino-Rey, Alnaief, Zetzl, & Smirnova, 2012; Griffin et al., 2014; Özbakır & Erkey, 2015; van Bommel & de Haan, 1995).

High pressure carbon dioxide (HPCD) techniques have been demonstrated as a valid alternative to heat pasteurization for inactivation of vegetative bacteria in liquid and solid foods (Ferrentino & Spilimbergo, 2011; Garcia-Gonzalez et al., 2007). Hence, drying with $scCO_2$ could provide dried products with reduced levels of contamination. However, it has been shown that a reduced water activity can decrease the effect of HPCD on microorganisms (Calvo & Torres, 2010; Ferrentino & Spilimbergo, 2011). As a consequence, there is a need to investigate microorganisms' survival throughout $scCO_2$ drying to determine if this technology has the potential to serve as a hybrid drying-decontamination technique for medium to high value-added products such as herbs, berries and spices.

Therefore, the aim of this study was to examine the applicability of $scCO_2$ drying to obtain safe dried coriander leaves and stems. Coriander was chosen for its commercial value and its high initial contamination level (FDA, 2001). This work studied the effect of $scCO_2$ treatment and consecutive drying on the natural microbiota of coriander and on inoculated pathogens: *Salmonella enterica* subsp. *enterica*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7. Inactivation of the natural microbiota and the inoculated pathogens was further compared with the inactivation obtained when freeze-drying was applied instead of $scCO_2$ drying.

2. Materials and methods

2.1. Material

Fresh coriander (*Coriandrum sativum*) was purchased from a local retailer in Ghent, Belgium, and kept at 4 °C prior use for maximum three days. The product origin was variable and depended on the period of the year.

2.2. Bacterial strains

Three strains of *E. coli* O157:H7 (ATCC 700728, BRMSID 188, LFMFP 846), *L. monocytogenes* (LMG 23192, LMG 23194, LMG 26484) and *S. enterica* (serovars Thompson RM1987 and Typhimurium SL1344, LFMFP 883) were used. All *E. coli* O157:H7 strains are *stx*-negative strains. LFMFP strains are part of the Research Unit Food Microbiology & Food Preservation culture collection at the Faculty of Bio-Science Engineering, Ghent University. LMG strains are part of the Belgian Coordinated Collection of Micro-organisms (BCCM) situated at the Laboratory of Microbiology at the Faculty of Sciences, Ghent University. *E. coli* O157:H7 LFMFP 846 is a nalidixic acid-resistant variant (50 μg/mL) of *E. coli* O157:H7 MB3885 (obtained from Prof. Piérard, EHEC Reference Laboratory at the University hospital in Brussels). *E. coli* O157:H7 BRMSID 188 was obtained from Dr. Susan Bach (Agri-Food Canada; Dinu & Bach, 2011). *E. coli* O157:H7 ATCC 700728 originated from a verocytotoxigenic strain which lost its ability to produce toxin. *Salmonella* Typhimurium (strain LFMFP 883) was an environmental isolate, provided from Prof. Venter from Pretoria University, South Africa and deposited to LFMFP-UGent culture collection. *Salmonella* (serovars Thompson RM1987 and Typhimurium SL1344) were obtained from Dr. Maria Brandl (ARS, USDA). The isolates of *E. coli* O157:H7, *L. monocytogenes*, and *S. enterica* subsp. *enterica* and their characteristics are summarized in Table 1. Before use, the strains stored on glass beads at −75 °C, were revived in 10 mL of Brain Heart Infusion (BHI, Oxoid, England) for 24 h at 37 °C. After 24 h, the strains were streaked on Tryptic Soya Agar (TSA, Oxoid) and on an appropriate selective medium (Table 1). After 24 h, one colony was taken from the selective medium and TSA slants were inoculated and incubated for 24 h at 37 °C. These working cultures were stored at 4 °C for up to 1 month.

2.3. Inoculation

For each experiment, one cocktail containing three strains of a given species was prepared. The working inoculum was prepared by individually culturing the strains from the slants in 10 mL of BHI for 24 h at 37 °C. One milliliter of the culture was transferred into a 2-mL Eppendorf and centrifuged (2900 × *g* for 10 min). The supernatant was discarded and the pellet was re-suspended in 1 mL of peptone physiological salt solution (PPS) prepared with 1 g/L of neutralized bacteriological peptone (Oxoid) and 8.5 g/L of NaCl (Merck, Germany). For each strain, this washing step was repeated twice. The three pellets, each containing one of the three strains, were resuspended in 0.5 mL of PPS and transferred into a 2-mL Eppendorf tube. Finally, 0.5 mL of PPS was added to obtain a final volume of 2 mL. For each microorganism,

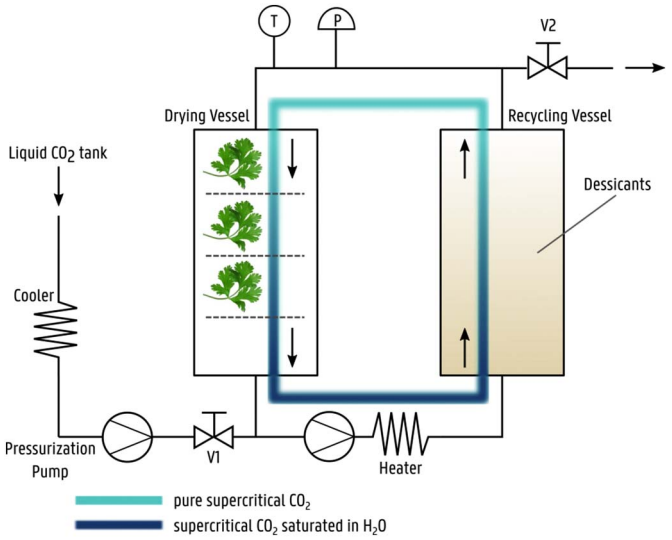

Fig. 1. Process flow diagram of the supercritical CO_2 dryer. Pure CO_2 is cooled to 5 °C and fed by a pressurization pump to the drying system. In the drying system, pressure and temperature of the CO_2 are increased above their critical values (80–100 bar; 35–40 °C) in a closed loop. In the drying vessel, water is extracted from the food product and carried away by the supercritical CO_2 to the recycling vessel. There, zeolites (solid desiccants) remove water from the CO_2 flow and a new cycle begins. The flow of CO_2 inside the drying vessel is forced by a circulation pump.

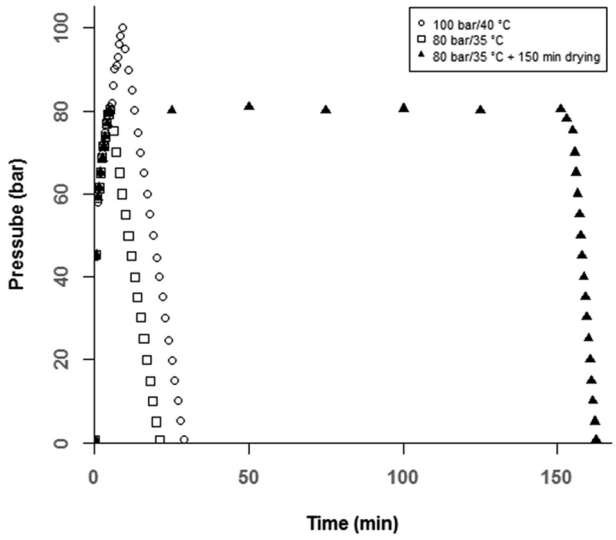

Fig. 2. Pressure profile of the different supercritical CO_2 treatments: 100 bar/40 °C/no drying (○), 80 bar/35 °C/no drying (□); 80 bar/35 °C/drying for 150 min (▲).

the inoculation cocktail contained the three isolates shown in Table 1. The average microbial counts in the cocktails were 8.74 ± 0.17, 8.50 ± 0.50, and 9.19 ± 0.28 CFU/mL for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively.

Coriander leaves and stems were aseptically cut in pieces of 2 cm and placed in sterile plastic trays. The trays contained 25 or 50 g for controls and samples, respectively. The specimens were spot inoculated by means of 5 µL of inoculation cocktail per g of product to obtain an inoculum concentration of approximately 6 log CFU/g. The droplets were deposited in different locations and coriander was regularly mixed to ensure homogeneity. After inoculation, the samples were left for 1 h to dry in a bio-safety cabinet allowing the microorganisms to attach to the surface.

2.4. Supercritical CO_2 treatment

$ScCO_2$ treatments were performed in the equipment schematically presented in Fig. 1 according to the description made in Agterof, Batia, and Hofland (2012). Liquid food grade CO_2 was fed into a high pressure system (property of FeyeCon, The Netherlands) by a high pressure pump. The drying system consisted of a 1-L drying vessel containing the product, a 2-L recycling vessel hosting zeolites (Zeolite 3A, Zeochem, Switzerland) and a circulation pump. The temperature of the system was controlled by a water bath.

For one treatment, one sample was divided and loaded into four stainless steel baskets (the sample was too large to fit in only 1 or 2 baskets). After the treatment, the contents of the baskets were pooled again into one sample. Three treatments were considered for this study: 80 bar at 35 °C and 100 bar at 40 °C, both followed by consecutive depressurization at 5 bar/min, and 80 bar at 35 °C for 150 min of drying, followed by depressurization at 5 bar/min. The treatment time and the pressure profile can be seen in Fig. 2. After each treatment, the vessel was filled by a mix of ethanol and water (7:3) for 10 min and then flushed with pure CO_2 for 3 min; the stainless steel baskets were autoclaved in between each treatment.

Each experiment included one $scCO_2$ treated inoculated sample, one inoculated control sample, and one non-inoculated control sample and was performed in triplicate.

2.5. Freeze-drying treatment

Freeze-drying was performed in a laboratory freeze-dryer (Alpha 1-2 LD, Christ, Germany). After inoculation, the samples were frozen at −80 °C overnight. Then they were freeze-dried at 0.012 bar for 23.5 h, followed by 30 min at 0.007 bar. The temperature of the condenser was kept at −55 °C throughout the drying. Each experiment included 1 freeze-dried inoculated sample, 1 frozen inoculated control sample, 1 fresh inoculated control sample, and 1 non-inoculated control sample and was performed in triplicate.

2.6. Microbial analyses

The samples were retrieved in a sterile filter stomacher bag (BagFilter, Interscience, France) and diluted 1:10 in Buffered Peptone Water (BPW, Oxoid), followed by 1 min stomaching. Dried products were left for 15 min so that they could rehydrate and frozen samples were left to thaw at room temperature before stomaching. After stomaching, 10-fold dilutions were prepared.

2.6.1. Enumeration of the natural microbiota

Total aerobic count was assessed on Plate Count Agar (PCA, Oxoid) at 30 °C for 48–72 h, yeasts and molds on Yeast Glucose Chloramphenicol agar (YGC, Bio-Rad, France) at 22 °C for 72 h, and Enterobacteriaceae on Rapid'Enterobacteriaceae (REB, Bio-Rad) at 37 °C for 24–48 h. Moreover, to determine the total aerobic mesophilic spores count, the first dilution was first submitted to a thermal treatment of 80 °C for 10 min followed by enumeration on PCA after incubation at 30 °C for 48–72 h.

2.6.2. Enumeration of the inoculated pathogens

The appropriate dilutions were spread-plated on Cefixime-Tellurite Sorbitol MacConkey Agar (CT-SMAC, Oxoid) containing nalidixic acid (50 µg/mL, Sigma-Aldrich, Germany) and CT-SMAC containing kanamycin (100 µg/mL, Sigma-Aldrich) for *E. coli* O157:H7. *Salmonella* was enumerated on Xylose Lysine Deoxycholate (XLD, Oxoid) containing nalidixic acid (50 µg/mL), XLD containing kanamycin (100 µg/mL), and XLD containing streptomycin (100 µg/mL, Sigma-Aldrich). *L. monocytogenes* was enumerated on Agar *Listeria* Ottavani and Agosti (ALOA, BioLife Italiana, Italy). CT-SMAC and XLD agars were supplemented with antibiotics because preliminary experiments showed that the natural microbiota present in coriander caused interference in the enumeration (results not shown).

To reduce the supplementary stress of the antibiotics on injured cells, a TSA layer was poured on top of the plates, as suggested by Kang and Fung (2000). To lower the detection limit to 10 CFU/g on the spread-plates, 1 mL of the first dilution was spread over 3 different plates. The incubation was performed at 37 °C for 24 h for *E. coli* O157:H7 and *Salmonella*, and at 37 °C for 48 h for *L. monocytogenes*.

2.6.3. Detection of the inoculated pathogens

The stomacher bags that contained the first dilution in BPW were incubated for 24 h at 37 °C to serve as an unselective enrichment. After incubation, the enriched samples were plated on an appropriate medium cited above. When no growth was observed on the plates after enrichment, the given microorganisms count was indicated as below 1 CFU/50 g of fresh product.

2.7. Data analyses

Counts were expressed in $\log_{10}$ CFU per g and normalized per g of fresh product to remove the bias induced by the mass loss occurring during treatment. The conversion was performed by multiplying the counts in CFU/g by the mass of the treated sample divided by the mass of the fresh product.

Reductions are expressed as $\log_{10}(N_0/N)$ where N_0 was the number of initial microorganisms in the untreated sample and N the number of viable microorganisms after the treatment, in CFU/g of fresh product. Results were analyzed with one-way analysis of variance to compare effects of the different treatments with significance at $\alpha = 0.05$.

3. Results and discussion

3.1. Effect on the natural microbiota

Fresh coriander had a water activity of 0.99. Freeze-drying and $scCO_2$ drying both resulted in dried coriander with a water activity of 0.20 ± 0.04 and 0.21 ± 0.02, respectively. The corresponding weight losses were 88.23 ± 0.87 and 89.13 ± 0.53% for $scCO_2$ drying and freeze-drying, respectively. Table 2 displays the counts of native microbiota obtained after freeze-drying and $scCO_2$ drying of coriander. The fresh coriander was mainly contaminated with Enterobacteriaceae with 7.07 ± 0.60 and 6.18 ± 0.55 CFU/g for the $scCO_2$ drying experiments and for the freeze-drying experiments, respectively.

3.1.1. Effect of $scCO_2$ drying

With $scCO_2$ drying, the total aerobic plate count was reduced by 2.80 ± 0.58 log CFU/g whereas mesophilic aerobic spores were not significantly affected by the treatment ($p > 0.05$). However, the surviving fraction of mesophilic bacteria cannot originate solely from the germinated spores. The number of mesophilic spores after the treatment was 2.79 ± 0.1 log CFU/g whereas the total aerobic plate count was 4.51 ± 0.67 CFU/g for. Therefore, it can be concluded that a part of the vegetative bacteria also survived the $scCO_2$ drying process. The weak effect of HPCD on bacterial spores in liquid media has already been reviewed (Damar & Balaban, 2006; Garcia-Gonzalez et al., 2007). According to Garcia-Gonzalez et al. (2007), HPCD treatments alone at mild temperature (20–40 °C) and moderate pressure (< 150 bar) are insufficient for significant spores reduction.

Enterobacteriaceae were reduced by 4.61 ± 0.40 log CFU/g and were always detected in the samples after $scCO_2$ drying (> 100 CFU/g). These results are consistent with those of Ferrentino, Balzan, Dorigato, Pegoretti, and Spilimbergo (2012) who showed a reduction from 4.90 ± 0.13 to 1.58 ± 0.17 CFU/g of the total coliforms on fresh-cut coconut after treatment at 35 °C and 120 bar for 60 min. In their study, an increase to 45 °C at 120 bar was enough to reduce the coliforms by > 4 log CFU/g in 15 min. In this study, Enterobacteriaceae were less resistant to $scCO_2$ drying than the rest of the mesophilic bacteria.

Yeasts and molds were absent in all samples with a log reduction higher than 5.03. This high sensitivity of yeasts and molds to HPCD has already been reported. Liao, Zhang, Hu, and Liao (2010) reported a complete inactivation of yeasts and molds after a 30-min treatment at 20 MPa above 42 °C in apple juice. They also reported a stronger resistance of the aerobic bacteria to HPCD with a complete inactivation occurring at 20 MPa at 62 °C for 30 min. Similarly, Parton, Elvassore, Bertucco, and Bertolonic (2007) found that *Saccharomyces cerevisiae* inoculated in industrial pear juice was reduced by > 6.39 log CFU/g when treated by HPCD at 38 °C and 9 MPa. On fresh-cut pears inoculated with *S. cerevisiae*, Valverde, Marín-Iniesta, and Calvo (2010) observed a complete inactivation after a 2 to 6 min treatment at 55 °C regardless of the pressure used in the range 60–300 bar. In this study the treatment time (150 min) was significantly longer which could explain the complete inactivation obtained at the lower temperature of 35 °C.

3.1.2. Effect of freeze-drying

Freeze-drying is one of the preferred methods to prepare stock cultures of microorganisms (Morgan, Herman, White, & Vesey, 2006). Therefore, it is expected that this technology has a mild effect on them. In this study, the aerobic plate count, Enterobacteriaceae, and yeasts and molds were significantly reduced by 1.23, 0.87 and 0.97 log CFU/g, respectively ($p < 0.05$). The decrease in mesophilic spores was not significant ($p > 0.05$).

3.1.3. Comparison $scCO_2$ drying versus freeze-drying

In terms of inactivation of the natural microbiota, freeze-drying has a milder effect on microorganisms than $scCO_2$ drying, except on mesophilic spores, on which both technologies were ineffective. These results emphasize that although freeze-drying brings the highest quality when organoleptic properties are concerned, a high number of spoilers may still be present on the product after dehydration. On the contrary, the application of $scCO_2$ drying significantly increased the reduction of the natural microbiota (except the spores) with a complete inactivation of the yeasts and molds. Moreover, $scCO_2$ drying was able to reduce the Enterobacteriaceae by > 4 log CFU/g. Since these microorganisms are often used as hygiene indicators (Reij & Den Aantrekker, 2004), this technology could be used for products with a high initial contamination level, such as coriander, parsley and other herbs. However, since $scCO_2$ is known as an extraction medium, there is a need to investigate the

Table 2
Initial counts and reductions after supercritical CO_2 drying at 80 bar and 35 °C for 150 min and after freeze-drying (0.012 bar/23.5 h + 0.007 bar/0.5 h), and freezing at −80 °C overnight (means ± standard deviations, in log CFU/g of fresh product; n = 3).

Microorganisms	Supercritical drying		Freeze-drying		
	Initial count	Log reduction after drying	Initial count	Log Reduction after freezing at −80 °C	Total log reduction after freeze drying
Aerobic plate count	7.31 ± 0.65	2.80 ± 0.58	6.90 ± 0.28	0.30 ± 0.26	1.23 ± 0.43
Mesophilic spores	2.90 ± 0.32	0.11 ± 0.33	2.97 ± 0.28	−0.01 ± 0.10	0.6 ± 0.36
Enterobacteriaceae	7.07 ± 0.60	4.61 ± 0.40	6.18 ± 0.55	0.26 ± 0.33	0.87 ± 0.35
Yeasts and molds	6.09 ± 0.05	> 5.03[a]	5.32 ± 0.46	0.23 ± 0.41	0.97 ± 0.74

[a] Inactivation below 10 CFU/g of fresh product.

Table 3
Initial count of *E. coli* O157:H7, *Salmonella* and, *L. monocytogenes*, inoculated on coriander and inactivation after different supercritical CO_2 treatments (means ± standard deviations, in log CFU/g of fresh product; n = 3).

Conditions	Microorganism	Initial count	Log reduction
Pressure: 80 bar	*Escherichia coli* O157:H7		
Temperature:	BRMSID 188	5.60 ± 0.49	4.36[a]
35 °C	NCTC12900 & LFMFP	5.62 ± 0.40	4.33[a]
Drying time: 0 min	846		
	Salmonella		
	S. Thompson RM1987	5.89 ± 0.12	4.92[a]
	S. Typhimurium SL	6.00 ± 0.24	4.84 ± 0.45
	1344		
	S. Typhimurium	5.96 ± 0.23	5.00 ± 0.03
	LFMFP 884		
	Lsteria monocytogenes		
	LMG 23192. LMG	6.37 ± 0.19	4.46 ± 0.63
	23194 & LMG 26484		
Pressure: 100 bar	*Escherichia coli* O157:H7		
Temperature:	BRMSID 188	5.67 ± 0.38	7.37[b]
40 °C	NCTC12900 & LFMFP	6.05 ± 0.41	7.75[b]
Drying time: 0 min	846		
	Salmonella		
	S. Thompson RM1987	5.73 ± 0.23	4.77[a]
	S. Typhimurium SL	5.82 ± 0.18	4.85[a]
	1344		
	S. Typhimurium	5.70 ± 0.21	4.73[a]
	LFMFP 884		
	Listeria monocytogenes		
	LMG 23192. LMG	6.37 ± 0.03	4.99 ± 0.39
	23194 & LMG 26484		
Pressure: 80 bar	*Escherichia coli* O157:H7		
Temperature:	BRMSID 188	5.70 ± 0.13	5.29[a]
35 °C	NCTC12900 & LFMFP	5.58 ± 0.36	5.18[a]
Drying time:	846		
150 min	*Salmonella*		
	S. Thompson RM1987	6.06 ± 0.23	5.69 ± 0.15
	S. Typhimurium SL	6.00 ± 0.46	5.47 ± 0.11
	1344		
	S. Typhimurium	5.93 ± 0.38	5.32 ± 0.50
	LFMFP 884		
	Listeria monocytogenes		
	LMG 23192. LMG	6.69 ± 0.12	5.55 ± 0.42
	23194 & LMG 26484		

[a] Inactivation below the enumeration limit (150 CFU/g of fresh product).
[b] Not detected after enrichment (1 CFU/50 g of fresh product).

quality of the product afterwards. In this study, the quality of dried coriander was assessed visually and by smell and coriander dried from both techniques could not be distinguished. Some studies have shown that $scCO_2$ drying is better than air-drying to keep organoleptic properties (Brown et al., 2008; Bušić et al., 2014). In particular, Bušić et al. (2014) found that freeze-drying was best for the preservation of color and bioactive compounds of basil, followed by $scCO_2$ drying while air-drying had the most adverse effect. Nevertheless, deeper studies on sensory properties and consumer acceptability should be performed to compare products dried with these different technologies.

3.2. Effect on the inoculated pathogens

3.2.1. Effect of $scCO_2$ treatment and drying

Regardless of the conditions, $scCO_2$ treatments induced significant inactivation ($p < 0.05$) for all strains (Table 3). At 80 bar and 35 °C, all *E. coli* O157:H7 strains were reduced below the quantification limit (150 CFU/g of fresh product) which indicates that, under the same conditions, this microorganism is less resistant than the tested *Salmonella* and *L. monocytogenes* strains. *S.* Thompson RM1987 was the only strain of *Salmonella* reduced below the quantification limit under these conditions. Because *L. monocytogenes* strains could not be distinguished from each other on the growing medium, it cannot be stated if any of the tested strains is less resistant than the others. The reductions obtained after $scCO_2$ drying could also be partially caused by detachment of the microorganisms from the surface. However, in the case of *E. coli* 157:H7, a complete detachment from the surface after treatment at 100 bar and at 40 °C is highly unlikely. Moreover, swabs were taken inside the dryer between experiments and tested for presence of the inoculated strains of *E. coli* O157:H7, and the strains were never found. It can therefore be concluded that, although detachment is possible, the observed reductions can be attributed to an actual inactivation and not detachment.

Increasing the pressure from 80 to 100 bar and temperature from 35 °C to 40 °C induced significant changes in inactivation of *E. coli* O157:H7 and *Salmonella*. Indeed, *E. coli* O157:H7 could not be detected in the samples after enrichment indicating a reduction of > 7 log CFU/g. These results are consistent with those of Ferrentino, Calliari, Bertucco, and Spilimbergo (2014) on carrots spiked with *E. coli* O157:H7. They reported 7 log reduction after treatment for 6 min at 120 bar and 35 °C. In liquid medium, Kim, Rhee, Kim, and Kim (2007) showed that *E. coli* O157:H7 ATCC 35150 and 25,922 were reduced by 8 log CFU/mL in cell suspension after treatment at 80–150 bar at 40 °C for 20 min. *Salmonella* strains were reduced below the quantification limit but were still detected in the enriched samples. This means that the effective reduction lies between 4.73 and 6.51 log CFU/g. The increased efficiency of this treatment against *Salmonella* and *E. coli* O157:H7 could be explained by the increase in temperature and/or in pressure. These results are in agreement with Kim, Rhee, Kim, Lee, and Kim (2007) who found that a 35–45 °C temperature range increase enhanced significantly the inactivation of *S.* Typhimurium ATCC 700408 at 100 bar in physiological saline and phosphate-buffered saline. No significant difference could be observed for *L. monocytogenes* between the 2 treatments. *L. monocytogenes* exhibited the stronger resistance for all treatments. According to Garcia-Gonzalez et al. (2007), Gram-positive bacteria were in most cases reported to be more resistant than Gram-negative bacteria during HPCD decontamination.

It was previously reported that the water activity can influence the effect of HPCD on inactivation of microorganisms (Dillow, Dehghani, Hrkach, Foster, & Langer, 1999). Therefore, drying could impair inactivation. In this study, the application of a 150 min drying time at 80 bar/35 °C resulted in a dried coriander but did not induce higher quantifiable inactivation. Although the treatment with and without drying resulted in similar inactivation values, it was not possible to discriminate between the effect of the drying time and the decrease in water activity with the current experimental setup. To the knowledge of the authors, this is the first study to assess the microbial inactivation during drying with supercritical CO_2, hence comparison with other studies is not possible. Nevertheless, these results indicate potential of $scCO_2$ drying to obtain dried coriander with significantly reduced levels of contamination.

3.2.2. Effect of freeze-drying

Reductions of the pathogenic strains after freezing at −80 °C and freeze-drying are presented in Table 4. Freezing at −80 °C overnight had no or a relatively mild effect on the tested strains. Indeed, *L. monocytogenes* strains were not significantly reduced after freezing at −80 °C ($p > 0.05$) whereas the *Salmonella* and *E. coli* O157:H7 strains were all significantly but only slightly reduced by 0.47 to 0.79 log CFU/g. After freezing followed by drying, the total reduction was significant for *Salmonella* and *E. coli* O157:H7 ($p < 0.05$), and for *L. monocytogenes* to a lower extent ($p < 0.05$). Freeze-drying increased the reduction to approximately 1.5 and 2 log CFU/g for *E. coli* O157:H7 and *Salmonella*, respectively. The high resistance of *L. monocytogenes* to the freezing process was already observed for meat and fresh fruits and vegetables at temperatures of −18 to −20 °C (Flessa, Lusk, & Harris, 2005; Palumbo & Williams, 1991). Miyamoto-Shinohara, Sukenobe, Imaizumi, and Nakahara, (2006) investigated the survival of 2 Gram-positive microorganisms, namely *Enterococcus faecium* (5 strains) and *Lactobacillus acidophilus* (8 strains), 3 Gram-negative, *E. coli* (144 strains),

Table 4
Initial count (log CFU/g) of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, inoculated on coriander and inactivation after freeze-drying (0.012 bar/23.5 h + 0.007 bar/0.5 h) and freezing at −80 °C overnight (means ± standard deviations, in log CFU/g of fresh product; n = 3).

Microorganism	Initial count	Log reduction after freezing at − 80 °C	Total log reduction after drying
Escherichia coli O157:H7			
BRMSID 188	5.37 ± 0.58	0.47 ± 0.01	1.43 ± 0.26
NCTC12900 & LFMFP 846	5.35 ± 0.62	0.79 ± 0.03	1.53 ± 0.33
Salmonella			
S. Thompson RM1987	5.86 ± 0.23	0.71 ± 0.42	1.91 ± 0.46
S. Typhimurium SL 1344	5.77 ± 0.06	0.74 ± 0.38	1.89 ± 0.39
S. Typhimurium LFMFP 884	5.63 ± 0.15	0.78 ± 0.09	2.03 ± 0.32
Listeria monocytogenes			
LMG 23192, LMG 23194 & LMG 26484	6.52 ± 0.19	−0.16 ± 0.15	0.71 ± 0.34

Pseudomonas putida (45 strains), and *Enterobacter cloacae* (11 strains), and 1 yeast, *Saccharomyces cerevisiae* (102 strains). They found that Gram-positive bacteria had the highest survival rate immediately after freeze-drying, followed by the Gram-negative and finally by *S. cerevisiae*. The better resistance of Gram-positive strains to freeze-drying could explain the reason why *L. monocytogenes* resisted better in this study. In their study, *E. coli* strains had an average survival of 42.6% which represents a lower reduction than that of the 3 strains tested here. This difference could be attributed to the fact that they used a suspension medium with protective compounds (10% skimmed milk and 1% sodium glutamate) whereas in this study the bacteria were deposited on a solid product.

3.2.3. Comparison $scCO_2$ drying versus freeze-drying

For every pathogen, $scCO_2$ drying was more efficient than freeze-drying to inactivate the inoculated bacteria. After freeze-drying, *L. monocytogenes* reduction did not exceed 0.71 ± 0.34 log CFU/g. For *Salmonella* and *E. coli* O157:H7, the reductions ranged from 1.43 to 2.03 CFU/g, which is far below the 5 to 5.5 log reductions obtained after $scCO_2$ drying.

Overall, these results showed that, product quality aside, $scCO_2$ drying could be a potential alternative to freeze-drying to provide dried coriander with a reduced number of pathogenic and spoilage microorganisms. Moreover, the reductions obtained in this study after $scCO_2$ drying are considerably higher than the reductions obtained after drying of fresh fruits, vegetables and herbs reviewed in Bourdoux et al. (2016) with different drying techniques. The European Chilled Food Federation (ECFF, 2006), recommends aiming at 6 log reduction of *L. monocytogenes* for chilled food products. After treatment at 80 bar/35 °C for 150 min, *L. monocytogenes* was reduced by 5.55 log CFU/g. Although this does not meet the ECFF recommendation, the end product is dried and therefore there is no risk of growth prior to rehydration. Moreover there is room to improve the treatment to obtain higher inactivation. Indeed, use of co-solvent, higher pressure, higher temperature, and longer treatment time could increase the resulting inactivation. For *Salmonella*, a Quantitative Microbial Risk Assessment on almonds showed that a minimal reduction of 4 log CFU/g would result in an estimated mean risk of illness below one case per year in the United States (Santillana Farakos et al., 2017). After treatment at 80 bar/35 °C for 150 min, *Salmonella* strains were reduced by > 5.3 log CFU/g which largely exceeds this recommendation. Finally, after $scCO_2$ drying *E. coli* O157:H7 strains were reduced below the limit of quantification (< 150 CFU/g of fresh product) with > 5 log reduction.

It was previously reported that *E. coli* and *L. monocytogenes* can build up resistance after multiple rounds of high pressure carbon dioxide treatment (Garcia-Gonzalez et al., 2010). Therefore, more research is needed to evaluate how quickly they acquire this resistance and to what extent it is relevant in actual processes. Also, a broader range of spoilage and pathogenic microorganisms from both Gram positive and Gram negative species should be investigated to elucidate difference in inactivation efficiency. Spore-forming bacteria, such as *Clostridium botulinum* and *Bacillus cereus*, inoculated on the fresh product prior to the drying would be of particular interest.

4. Conclusion

This study showed that $scCO_2$ drying can be a good alternative to freeze-drying of medium to high value added products. Although both techniques resulted in a dried product, freeze-drying could not reduce inoculated microorganisms to the recommended levels for *E. coli* O157:H7, *Salmonella* and *L. monocytogenes*. On the other hand, $scCO_2$ treatments were able to inactivate the same pathogens by 4.4 to > 7 log reductions. Amongst all microbial populations tested in this study, mesophilic aerobic spores and some mesophilic aerobic bacteria were not inactivated during the treatments. $ScCO_2$ was particularly effective on yeasts and molds which could not be detected in any sample after the treatment.

From a microbiology point of view, this technology offers obvious opportunities for the food industry. However, before this technology could be implemented in the food industry, the effects of the drying kinetics and the characteristics of the foods on microorganisms should be better understood. Finally, sensorial properties of the dried products should be thoroughly assessed after treatment and compared with a benchmark technology such as freeze-drying and/or air-drying.

Acknowledgments

This work was supported by European Union's Horizon 2020 research and innovation programme [grant number 653759]. The authors thank FeyeCon and CO_2 Dry which provided the supercritical CO_2 dryer.

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