



Review

Inactivation of parasite transmission stages: Efficacy of treatments on foods of non-animal origin

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ABSTRACT

Background: Among 24 foodborne parasites ranked by FAO/WHO, 15 are associated with food of non-animal origin (FoNAO). Control of these hazards is essential for food safety.

Scope and approach: Control measures to inactivate parasites in FoNAO are reviewed. Preventing contamination is key to ensuring the safety of fresh produce. However, additional control measures can further reduce the likelihood of occurrence of infectious parasites in FoNAO.

Key Findings and Conclusions: The efficacy of treatments depends on parasite species, developmental stage, matrix, and application conditions. Conventional pasteurization (72 °C; 15 s) inactivates parasites in most matrices, although some parasites are more heat resistant, and this may be an inappropriate method for many FoNAO that are intended for eating fresh and raw. Freezing at −20 °C for 2 days inactivates most, but not all, parasites, and some are highly resistant to freezing. Parasites generally survive chemical disinfection, making its application at effective doses often unsuitable at an industrial scale. Ozone and chlorine dioxide are the most promising in terms of efficacy and dosage, nevertheless challenges remain in their application especially for the most fragile produce. High-pressure processing is an efficient technology, providing good inactivation of parasites. Further research should focus on standardizing experimental approaches for evaluation of inactivation techniques and development of methods to measure parasite inactivation in food matrices.

1. Introduction and the growing awareness of food of non-animal origin as a vehicle for parasite infection

Foodborne diseases encompass a wide range of acute and chronic syndromes that differ greatly in their prevalence, duration, and severity. WHO estimates that 31 bacterial, viral, parasitic and chemical

global hazards caused a total of 600 million foodborne illnesses in 2010, of which 15% were due to parasites (WHO, 2015). Although fish and meat are often associated with biological hazards, foods of non-animal origin (FoNAO), such as fresh produce, are particularly associated with foodborne parasites (FBP) (Van Pelt et al., 2018). One third of domestically acquired parasitic foodborne illnesses in the US have

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been attributed to FoNAO (Painter et al., 2013); by extrapolation from the WHO estimates, this corresponds to around 30 million illnesses globally during 2010. Among the 24 FBP listed for risk-ranking by FAO/WHO in 2014 (FAO/WHO, 2014, p. 302) or the 25 FBP ranked by Bouwknegt et al. for Europe in 2016 (Bouwknegt et al., 2018), transmission of 15 of them, particularly those for which the transmission stage is shed in the feces of the definitive host, can be associated with FoNAO. Contamination of FoNAO may occur along the farm-to-fork continuum, either directly from infected handlers with poor hygiene (e.g., *Cryptosporidium* and *Cyclospora* oocysts, *Giardia* cysts, and *Ascaris* and *Taenia* eggs) or from feces of infected animals (e.g., *Cryptosporidium* and *Toxoplasma* oocysts, *Echinococcus* and *Toxocara* eggs), or indirectly via contaminated irrigation water, or use of sewage as fertiliser. Most FBPs have a low infective dose and robust transmission stages. Thus, implementing measures that prevent contamination with parasites during primary food production, by following Good Agricultural Practices, is generally preferable to relying upon removal/inactivation of parasite transmission stages in subsequent stages of the food chain (EFSA, 2018).

As various different types of fresh produce, particularly salad vegetables and fruits such as berries, are often consumed with minimal preparation, any measures implemented along the farm to fork continuum to prevent, remove, or inactivate parasites may provide a measure of protection of consumer health and should be considered in the frame of Hazard Analysis and Critical Control Points (HACCP) studies. With greater demand and current food trends advising consumers to increase their intake of fresh produce, in combination with global sourcing and improved transport chains, the possibility of fruit and vegetables contaminated with parasite transmission stages being distributed more widely may be increasing. The probability of introduction of new strains or species into non-endemic areas is also likely to rise. Control procedures in the fresh-produce chain mostly concern spoilage and pathogenic bacteria. However, whether those procedures affect the survival of parasite transmission stages has seldom been addressed. Furthermore, distinction between whether parasites on fresh produce have become non-viable (as assessed by a viability study) or non-infectious (as assessed using an infection model) is also of relevance, and seldom addressed. In this companion paper to an already published article addressing foods of animal origin (FoAO; Franssen et al., 2018), we provide an in depth review of treatments for inactivation of parasite transmission stages in FoNAO, emphasizing where data are insufficient and drawing attention to relevant new technologies.

2. Reference inclusion criteria

As with the FoAO companion paper (Franssen et al., 2018), a non-systematic literature review was used to gather relevant information from scientific publications, reports, and official documents. Articles were included that attempted to quantify parasite inactivation effects of different treatments for FoNAO. However, given the breadth of cover, addressing different parasites, different matrices, different inactivation methods, and different ways of assessing inactivation, it was difficult to ensure the quality of all the references. Including only those references that corresponded to our highest quality requirements (i.e., recent papers with detailed quantification of parasite inactivation using bioassay), some parasites, matrices, and different methods would have had no reference material and would thus have been excluded. Information from relevant articles was aggregated into a database that is presented in Tables 1–4.

3. Current state of knowledge

As with the article on FoAO (Franssen et al., 2018), we do not describe parasite biology, geographical distribution, disease in humans, relevance for trade, and impact on economically vulnerable populations

in this article. The relevant information on these aspects for parasites that may be transmitted via FoNAO is available in Annex 7 of the FAO/WHO multi-criteria based ranking for risk management of foodborne parasites (FAO/WHO, 2014, p. 302).

4. Avoiding contamination and key aspects of removal

Clearly, and from the HACCP perspective, prevention of contamination is essential in reducing transmission of parasites with FoNAO. Measures that may reduce contamination are ensuring that personnel in contact with FoNAO are not themselves infected, ensuring that appropriate hygiene facilities are available for farm workers, keeping animals out of food facilities, including at the farm level, using non-contaminated water within the food chain, from irrigation to washing prior to packing etc.

Should contamination occur, a proportion of parasites transmission stages may be removed from FoNAO by washing procedures without the requirement for an inactivation procedure. Generally, washing procedures in the fresh produce industry are intended to remove dirt, pesticide residues, and microorganisms responsible for quality loss (Gil, Selma, Lopez-Galvez, & Allende, 2009). Additionally, washing is used to pre-cool cut produce and remove cell exudates that may support microbial growth. Such procedures include spraying or deluging in water, and sometimes mechanical treatment of surfaces by brushes or spray washers, followed by rinsing; sanitizer treatments may also be involved. The water used for these purposes must also be clean (potable standard), so that it does not become a vehicle for contamination. Washwater quality, especially if recycled and not treated prior to reuse, is a concern of the fresh produce industry (Parish et al., 2003). Methods for sanitizing washwater are not the focus of this document and not considered further.

Although hardy items of fresh produce can be brush-washed, a process in which oscillating brushes are used to scrub surfaces for physical removal of soil and microorganisms (Parish et al., 2003), this is not suitable for fresh produce such as lettuce and soft fruit. Addition of particles to washwater to increase abrasion is impractical, as additional wastewater treatment and particle recovery would be needed. However, introducing air into liquid provides additional cleaning forces and modern aeration ‘jacuzzi’ washers reduce bacterial loads on vegetables by between 1 and 2 logs (Gil et al., 2009). Since bacteria are smaller than parasite transmission stages, it can be speculated that shear forces should be larger, and thus the parasites would be easier to remove, and use of this technology should be explored regarding parasites. However, the transmission stages of many parasites, are “sticky”, and thus may be more difficult to remove than bacteria. It should also be noted that various characteristics of the surfaces of the fresh produce (cracks, crevices, hydrophobic tendency, texture etc.) may affect the ability of the washing procedure to remove adherent parasites.

Despite removal of bacteria from fresh produce by washing being given considerable attention, investigations on removal of parasites from fresh produce surfaces by washing procedures are largely lacking. Among helminths, the adhesive properties of e.g. *Taenia* eggs (OIE, 2018), *Echinococcus* eggs (Eckert & Deplazes, 2004), and *Ascaris* eggs (Jimenez, 2007) are well recognized. However, one older study found some washing approaches were successful at removing *Fasciola* metacercaria from leafy greens (el-Sayad, Allam, & Osman, 1997).

Studies on removal of protozoa from the fresh produce surfaces are also sparse, but removal of *Cryptosporidium* oocysts from the surface of apples has been explored (Macarisin, Santin, Bauchan, & Fayer, 2010). None of the washing methods tested removed all oocysts from apple peel, with the most efficient removal achieved either by rigorous manual washing in water with a detergent or agitation in an orbital shaker with tris-sodium dodecyl sulfate buffer. Scanning electron microscopy revealed that some oocysts were in deep natural crevices in the apple exocarp, and some oocysts were closely associated with an amorphous substance with which they appeared to be attached to the

Table 1
Effects of chemical inactivation methodologies on foodborne parasites. Control measures: chlorine dioxide, hydrogen peroxide.

Transmission stage	Condition	Method for determining inactivation/ viability	Effect	Log reduction	Matrix	Ref
CHLORINE DIOXIDE <i>Cryptosporidium</i> oocysts	CT = 82 mg min/L. Spot inoculation; vegetables placed in a chamber in presence of gas	Cell culture assay	2.60 Log reduction (Log MPN/g) of oocysts infectivity	2.60	basil	Ortega et al. (2008)
			3.31 Log reduction (Log MPN/g) of oocysts infectivity	3.31	lettuce	
HYDROGEN PEROXIDE <i>Cryptosporidium</i> oocysts	0.03% H ₂ O ₂ ; for 1 h; acids added to juices in granular form on a weight/volume basis	Excystation	0.33 log reduction in oocysts excystation	0.33	apple cider	Kniel et al. (2003)
			0.44 log reduction in oocysts excystation	0.44	orange juice	
			0.48 log reduction in oocysts excystation	0.48	purple grape juice	
			0.47 log reduction in oocysts excystation	0.47	white grape juice	
		Cell culture	3.5 log reduction of oocysts infectivity	3.50	apple cider	
			4.4 log reduction of oocysts infectivity	4.40	orange juice	
		Excystation	4.1 log reduction of oocysts infectivity	4.10	purple grape juice	
			4.0 log reduction of oocysts infectivity	4.00	white grape juice	
			0.55 log reduction in oocysts excystation	0.55	purple grape juice	
			0.46 log reduction in oocysts excystation	0.46	apple cider	
Cell culture	> 5.9 log reduction of oocysts infectivity	> 5.90	orange juice purple grape juice			
0.03% H ₂ O ₂ ; for ≥ 2 h; acids added to juices in granular form on a weight/volume basis	Excystation	3 log reduction of oocysts infectivity	3.00	white grape juice apple cider		
		0.60 log reduction in oocysts excystation	0.60	purple grape juice		
	Cell culture	0.56 log reduction in oocysts excystation	0.56	apple cider		
		0.53 log reduction in oocysts excystation	0.53	orange juice		
		0.54 log reduction in oocysts excystation	0.54	white grape juice		
		≥5.9 log reduction of oocysts infectivity	5.90	orange juice purple grape juice white grape juice apple cider		

Table 2
Effects of thermal inactivation methodologies on foodborne parasites. Control measure: heat treatment.

Transmission stage	Condition	Method for determining inactivation/viability	Effect	Log reduction	Matrix	Ref
<i>Cryptosporidium</i> oocysts	70 °C; 5 s; 71.7 °C; 5 s; ≥ 70 °C and above; ≥ 10 s	semiquantitative in vitro infectivity assay: bovine kidney cells infected with heat-treated oocysts, followed by examination by indirect fluorescent antibody staining	3 log reduction of oocysts infectivity 4.8 log reduction of oocysts infectivity > 4.9 log reduction of oocysts infectivity	3.00 4.80 4.90	apple cider	Deng and Cliver (2001)
	Microwave heating; 2450 MHz; 850 W; 5 min	propidium iodide (PI) staining	93.23% oocysts PI positive in average (6 replicates)	1.19	green pepper	Duhain et al. (2012)
	200 ppm; 40 s washing + microwave heating; 2450 MHz; 850 W; 5 min Blanching; 96 °C; 3 min		98.10% oocysts PI positive in average (6 replicates) 93.53% oocysts PI positive in average (6 replicates)	1.72 1.17		
	<i>Cyclospora cayentanensis</i> oocysts	Water bath, minimum internal berry temperature of 80 °C maintained for 1 h; (<i>Eimeria acervulina</i> used as a surrogate) 70 °C; 15 min 100 °C; 15 min	bioassay: heat-treated oocysts fed to chicks, followed by evaluation of duodenal lesions or oocysts in cecal contents or in fecal contents percentage of sporulation as an indicator of viability	Absence of <i>E. acervulina</i> oocysts in feces of chicks, at 6 days postinoculation. Initial concentration between 400 and 650 oocysts. 0% sporulation; counts made on counting cell on 100–150 organisms - at least 2 log inactivation	n.s.	raspberries
<i>Echinococcus</i> eggs	25 °C; in air of 27% relative humidity; 48 h	bioassay: voles orally infected with eggs	eliminates infectivity for voles, 3 voles inoculated with eggs, none infected	n.s.	in vitro	Veit et al. (1995)
	43 °C; suspended in water; 4 h		eliminates infectivity for voles, 7 voles inoculated with eggs, none infected			
	43 °C; in air of 15% relative humidity; 2 h		eliminates infectivity for voles, 8 voles inoculated with eggs, none infected		basil	Sathyanarayanan & Ortega (2006)
	45 °C; in air of 85–95% relative humidity; 3 h		eliminates infectivity for voles, 3 voles inoculated with eggs, none infected eggs lose infectivity			Federer et al. (2015)
	65 °C; suspended in water; 180 min 70 °C; suspended in water; 30 min 75 °C; suspended in water; 15 min 80 °C; suspended in water; 7.5 min	sodium hypochlorite resistance test to assess in vitro egg viability at inoculation, then evaluation of infectivity of oncospheres by subcutaneous inoculation in mice				

n.s.: not stated.

Table 3
Effects of thermal inactivation methodologies on foodborne parasites. Control measure: freezing.

Transmission stage	Condition	Method for determining inactivation/viability	Effect	Log reduction	Matrix	Ref
<i>Cryptosporidium</i>	Exposure to 200 ppm chlorine; 40 s washing + Blast freezing: -20 °C; 4 min Blast freezing: -20 °C; 4 min, then thawing in 4 °C water for 3 min	propidium iodide staining	did not affect significantly the viability of the oocysts	-0.00	green pepper	Duhain et al. (2012)
<i>Cyclospora cayentanensis</i> oocysts	-18 °C; (<i>Eimeria acervulina</i> used as a surrogate of <i>Cyclospora</i>) -70 °C; 1 h -20 °C; 2 days	bioassay: heat-treated oocysts fed to chicks, followed by evaluation of duodenal lesions or oocysts in cecal contents or in fecal contents percentage of sporulation as an indicator of viability	0.10 log reduction of oocysts viability Absence of <i>E. acervulina</i> oocysts in feces of chicks, at 6 days postinoculation. Initial concentration between 400 and 650 oocysts. 0% sporulation; counts made on counting cell on 100–150 organisms - at least 2 log inactivation < 1% can sporulate; counts made on counting cell on 100–150 organisms - at least 2 log inactivation	n.s. 2.00	raspberries basil	Lee & Lee (2001) Sathyanarayanan & Ortega (2006)
<i>Echinococcus</i> eggs	-196 °C; 20 h -83 °C; 48 h	bioassay: voles orally infected with eggs	eliminates infectivity for voles, 10 voles inoculated with eggs, none infected eliminates infectivity for voles, 30 voles inoculated with eggs, none infected	n.s. n.s.	in vitro	Veit et al. (1995)
<i>Trypanosoma cruzi</i> trypomastigotes	-20 °C; 26 h	bioassay: trypomastigotes administered intraperitoneally, orally, or by gavage to immunodeficient mice/parasitemia quantified by Brener method	do not affect trypomastigotes infectivity for mice	0.00	açaí pulp	Barbosa et al. (2012)

n.s.: not stated.

Table 4
Effects of non-thermal inactivation methodologies on foodborne parasites. Control measure: drying, high pressure processing, gamma irradiation.

Transmission stage	Condition	Method for determining inactivation/viability	Effect	Log reduction	Matrix	Ref
DRYING						
<i>Giardia</i> cysts	Room temperature; 24 h	Vital dyes	Almost 50% die-off	0.30	lettuce	Uraaker et al. (2017)
HIGH PRESSURE PROCESSING						
<i>Cryptosporidium</i> oocysts	550 MPa; 30 s 550 MPa; ≥ 60 s 550 MPa; 30 s 550 MPa; ≥ 60 s	cell culture infectivity assay	gives 99,959% inactivation, reduction of oocysts infectivity gives > 99,992% inactivation, reduction of oocysts infectivity gives 99,993% inactivation, reduction of oocysts infectivity gives > 99,994% inactivation, reduction of oocysts infectivity	3.40 > 4.10 > 4.10 > 4.20	apple juice orange juice	Slifko et al. (2000)
<i>Cyclospora cayentanensis</i> oocysts	550 MPa; 2 min; 40 °C (<i>Eimeria acervulina</i> used as a surrogate of <i>Cyclospora</i>)	in vivo infection	eliminates oocysts infectivity for birds; no oocysts in feces, initial load 6 log	n.s.	raspberries basil	Knief et al. (2007)
<i>Toxoplasma</i> oocysts	340 MPa or above; 60 s	Mice bio-assay	eliminate infectivity for mice	n.s.	raspberries	Lindsay et al. (2008)
GAMMA IRRADIATION						
<i>Cyclospora cayentanensis</i> oocysts	1 kGy and above; (<i>Eimeria acervulina</i> used as a surrogate of <i>Cyclospora</i>)	Chicks bioassay	No duodenal lesions detected in natural host, no infectivity of <i>Eimeria acervulina</i> oocysts for chicks. Initial concentration between 400 and 650 oocysts.	n.s.	raspberries	Lee & Lee (2001)
<i>Toxoplasma</i> oocysts	0.5 kGy ≥ 0.2 kGy	Mice bioassay Cell culture plaque assay	kills oocysts on raspberries reductions of ≥ 4 log PFU/g	n.s. 4	raspberries Blueberries	Dubey et al. (1998) Lacombe et al. (2017)

n.s.: not stated.

apple surface. Another study demonstrated strong adherence of *Cryptosporidium* oocysts to spinach leaves, and also internalization of oocysts through the stomata, such that washing was ineffective for removal (Macarasin, Bauchan, & Fayer, 2010). In addition (Armon, Gold, Brodsky, & Oron, 2002), reports that *Cryptosporidium* oocysts are difficult to remove from the outer surfaces (skins) of courgettes (zucchini), possibly due to the hairs (trichomes) on the surfaces. Both apples and spinach are of relevance to *Cryptosporidium* transmission due to outbreaks of cryptosporidiosis associated with apple cider and with spinach leaves in salad.

Although no studies have directly investigated removal of *Cyclospora* oocysts from fresh produce by washing, an experiment in which *Eimeria acervulina*, a coccidian parasite of chickens, was used as a surrogate for *Cyclospora* on raspberries demonstrated that washing was generally inadequate (Lee & Lee, 2001). *Cyclospora* oocysts have been visualized attached to the surface of vegetables after washing and it has been reported that *Cyclospora* oocysts are “stickier” than *Cryptosporidium* oocysts, although the adhesins responsible for enhanced attachment have not been identified (Ortega & Sanchez, 2010). Experiments investigating removal of *Toxoplasma* oocysts from fresh produce by washing showed they adhere to berries, with greater attachment to raspberries than blueberries, presumably due to surface differences between the two berry types (Kniel et al., 2002).

5. Chemical inactivation methodologies

Although washing fresh produce with tap water can remove part of the indigenous microbiota, chemical disinfectants are usually added in the fresh produce industry to improve efficacy and to limit re-contamination of produce, the use of which depends on national regulations. Although washing with disinfectant decreases the concentration of microbial hazards, these strategies are insufficient to ensure microbiological safety of fresh produce, because the efficacy of the treatment will depend on several factors, including: i) the microbial target and initial load; parasite transmission stages are often more resistant than bacteria or fungi; ii) the conditions of application (type of disinfectant, concentration of disinfectant and contact time (CT value), pH, temperature); iii) the food matrix itself, which may protect the microorganism and/or modify the action of the chemicals. Thus, efficacy data obtained for pathogens in simple matrices (buffers, water) cannot be extrapolated to food matrices. Nevertheless, most studies concerning parasites only assess the efficacy of chemicals in simple matrices. Although the studies are informative, often the results cannot be directly transferred and applied to fresh produce.

5.1. Chemical oxidizers

5.1.1. Chlorine

Chlorine is the most widely used disinfectant in the fresh produce industry to sanitize food-processing environments and washwaters. Treatment efficiency depends on the concentration of free chlorine (i.e., hypochlorous acid, HClO) which varies with pH, temperature, exposure time, and the presence of organic matter. Although various studies have investigated the effect of chlorine on parasites (particularly *Cryptosporidium* and *Giardia*) in aqueous solutions, and generally found that very high CT are required to inactivate them, very few studies have investigated the effects on fresh produce. Only one study investigated the efficacy of chlorine at inactivating *Cryptosporidium parvum* oocysts inoculated onto green peppers, and an insignificant reduction in viability was reported with a CT of 67 mg min/L (100 mg/mL of free chlorine for 40 s), as determined by propidium iodide staining (Duhain, Minnaar, & Buys, 2012). In the minimally processed vegetable industries, chlorine is commonly used at concentrations of 50–200 ppm for 1–2 min, pH < 8.0 (CT from 50 to 400 mg min/L) and low temperatures (5–8 °C) (Goodburn & Wallace, 2013). Based on the experimental data available, these conditions would probably not result in

inactivation of parasites in FoNAO.

5.1.2. Chlorine dioxide

Chlorine dioxide (ClO₂) is a gas used for its powerful oxidizing properties. Table 1 presents results investigating the efficacy of chlorine dioxide to inactivate parasites on surfaces of vegetables or in various types of juice.

Ortega et al. (2008) found that basil and lettuce exposed to relatively low concentrations of gaseous chlorine dioxide for 20 min (CT of 82 mg min/L) led to 2.6–3.31 log reduction in infectivity of *Cryptosporidium* oocysts. Although high concentrations seem to be effective at controlling bacterial pathogens on apples, tomatoes, and onions without compromising their sensory quality, its use seems limited with other vegetables and berries because of the sensory impacts on the fresh produce. Although the infectious *Cryptosporidium* load was significantly reduced, *Cyclospora* sporulation was not affected. Thus, it was concluded that chlorine dioxide was not suitable as a sanitizer against parasitic contamination in the fresh produce industry (Ortega, Mann, Torres, & Cama, 2008).

5.1.3. Electrolyzed water

Electrolyzed water is a technique based on electrolysis of water containing sodium chloride to form hypochlorous acid and sodium hydroxide. The bactericidal effects have been tested on fresh produce by (Huang, Hung, Hsu, Huang, & Hwang, 2008), who found that disinfection efficacy may be reduced in the presence of organic matter due to formation of monochloramines. However, its efficiency at inactivating parasites on fresh produce is unknown.

5.1.4. Hydrogen peroxide and peroxyacetic acid

Hydrogen peroxide (H₂O₂) is a strong oxidizer, commonly used for disinfection purposes. Addition of hydrogen peroxide to fruit juices at concentrations as low as 0.025%–0.03% has been reported to result in a 25%–99.9% reduction of *C. parvum* infectivity, depending on duration of treatment, ranging from 1 to over 6 h. Exposure of *Cryptosporidium* oocysts to 0.03% H₂O₂ for 1 h resulted in 0.33–0.48 log inactivation in various fruit juices as measured by excystation, whereas a reduction of 3.5–4.4 log was measured by cell-culture infectivity (Kniel et al., 2003). Although exposure to hydrogen peroxide may inactivate *Cryptosporidium* oocysts, the exposure time and concentrations necessary seem unsuitable for treating FoNAO.

Peroxyacetic acid or peracetic acid (C₂H₄O₃) is a mixture of acetic acid (CH₃COOH) and hydrogen peroxide (H₂O₂) in aqueous solution that is used as a disinfectant in the food industry. However, its probable efficiency at inactivating parasite transmission stages on fresh produce seem low as (Briancesco, Veschetti, Ottaviani, & Bonadonna, 2005) found that peracetic acid (CT of 120 mg min/L) had only a marginal effect on reducing the viability of *Cryptosporidium* oocysts and *Giardia* cysts in effluent water.

5.1.5. Ozone

Ozone (O₃) is an inert gas with a high oxidant power and a short half-life (30 min at 15 °C, pH 7.0). The growing interest in ozone, rather than chlorine, for drinking water treatment is due to: i) its disinfection potential, enabling shorter contact times and lower concentrations; ii) its inactivation efficacy against a wide range of microbes; iii) the formation of relatively few toxic byproducts; iv) the possibility to treat large volumes of water. However, whether this can be extrapolated to FoNAO is less clear. Compared with chlorine, the disinfection rate of ozone is less affected by pH and temperature variations. However, ozone reacts rapidly with most organic and many inorganic compounds, decreasing persistence. Application of a high concentration for a short contact time is usually considered preferable. Although it has been used for reducing bacterial contamination of fresh produce for a considerable period, and is considered to be a safe option for use (Glowacz & Rees, 2016), to date, the efficacy of ozone at inactivating

parasites has been studied only in simple matrices such as water, and has not, to our knowledge been tested for its efficacy on parasites contaminating fresh produce.

5.2. Organic acids alone or in combination

Organic acids, such as acetic, malic, tartaric, or citric acids, have been tested as natural sanitizers (Uyttendaele, Neyts, Vanderswalmen, Notebaert, & Debevere, 2004). They are thought to inactivate cells by decreasing the pH, damaging membrane functions and key enzymes.

Acetic acid or vinegar is a natural, inexpensive readily available product with known sanitizing properties. However, little information exists on its efficacy at inactivating FBP, with only a few reports published. Zanini and Graeff-Teixeira (2001) investigated the larvicidal effect of wine vinegar on *Angiostrongylus costaricensis*, by incubating *A. costaricensis* L3 larvae at 20 °C for 1 h in red wine vinegar, with 1.5% (v/v) aqueous bleach solution and saturated cooking salt solution as controls and using mouse infectivity to evaluate inactivation. Incubation in vinegar resulted in a 1.25 log reduction in infectivity (Zanini & Graeff-Teixeira, 2001).

The efficacy of undiluted vinegar (4% v/v acetic acid) and diluted vinegar solutions at inactivating *G. duodenalis* cysts has been investigated (Costa, Thomaz-Soccol, Paulino, & Alcantara de Castro, 2009) Undiluted vinegar resulted in complete inactivation of the cysts following incubation for 60 min at 21 °C corresponding to 5.7 log reduction as measured by excystation. Thus, although very few studies have investigated the use of acetic acid/vinegar for inactivating FBP transmission stages on FoNAO, results available indicate that the efficacy depends on the organism, temperature, time, and concentration.

Citric acid, a major component of orange juice, has antimicrobial activity and has long been used in different food applications (Davidson, Sofos, & Branen, 2005). However, investigations on its ability to inactivate FBP are scarce and focus only on *Cryptosporidium*. The potential mechanisms and interactions between citric acid and *Cryptosporidium* oocysts have been discussed; an acidified environment may damage oocyst walls thereby reducing infectivity (Kato, Jenkins, Ghiorse, & Bowman, 2001; Kniel et al., 2003) (Friedman, Patten, Rose, & Barney, 1997). Although incubation in orange juice for 24 h at 4 °C failed to inactivate *C. parvum* oocysts completely, the methods used for determining viability gave conflicting results, with in vitro excystation indicating higher viability than dye staining. Kniel et al. (2003) tested the disinfection potential of organic acids, including citric acid, at inactivating *C. parvum* oocysts in juice. Citric acid was added to orange juice in concentrations ranging from 1 to 5% (w/v) at 4 °C and infectivity was measured in cell culture using human ileocecal adenocarcinoma cells. Infectivity decreased with increasing acid concentration, but complete inactivation was not achieved, even at the highest concentration. The addition of 5% citric acid resulted in a 23% decrease in *C. parvum* infectivity (Kniel et al., 2003). Dawson, Samuel, Scrannage, and Atherton (2004) investigated citric acid from 0.017 M at pH 7.0 to 0.089 M at pH 2.6 for inactivation of *C. parvum* oocysts. Viable sporozoites were detected after 14 days of contact time indicating a lack of efficacy at inactivating oocysts. Reductions in viability ranged from 17% at pH 7.0 (22 °C) to 52% at pH 2.6 (22 °C) or pH 7.0 (4 °C) (Dawson et al., 2004).

Levulinic acid is an inexpensive acid produced from renewable feedstocks (Bozell & Moens, 2000; Fang & Hanna, 2002) with known antimicrobial properties (Zhao, Zhao, & Doyle, 2009). It has been classified by the U.S. Food and Drug Administration as generally recognized as safe for direct addition to food as a flavoring substance or adjuvant (FDA, 2017). Ortega et al. (2011) evaluated the effects of levulinic acid alone or in combination with sodium dodecyl sulfate on the viability of *C. parvum* oocysts, using different contact periods from 30 min to 2 h and two concentrations (3% levulinic acid with 2% SDS and 2% levulinic acid with 1% SDS). Using cell culture to assess infectivity, the combination of 3% levulinic acid and 2% SDS decreased

oocyst viability by 0.41 log after 1 h of treatment (Ortega, Torres, & Tatum, 2011). These results suggest that levulinic acid and SDS are unlikely to be effective at inactivating *Cryptosporidium* oocysts on FoNAO, and other parasites that are more robust than *Cryptosporidium* are also likely to survive levulinic acid treatment.

Malic acid is an organic acid produced by all organisms and is partly responsible for the taste of fruits. Although known to inhibit fungi and bacteria, presumably due to pH alterations, studies on its effects on FBP are scarce. Kniel et al. (2003) investigated the effect of different concentrations (1, 3 and 5% w/v) of malic acid on *C. parvum* oocysts in apple cider, with oocyst viability evaluated by cell culture using HCT-8 cells. Infectivity reduction ranged between 0.11 log for 1% to 0.81 log for 5% (Kniel et al., 2003). Therefore, although malic acid is effective against bacteria and fungi, its effect on the viability of parasite transmission stages is probably insufficient in treating FoNAO against FBP.

As for the other organic acids, information on the effects of tartaric acid against FBP is scarce, with only one publication to date (Kniel et al., 2003). Tartaric acid is an organic compound occurring naturally in many plants and added to foods as an antioxidant. Again, antimicrobial properties of tartaric acid are associated with lowering of the pH (Davidson et al., 2005). The effect of tartaric acid on *C. parvum* oocyst viability was investigated by increasing the concentration of tartaric acid in purple and white grape juice (Kniel et al., 2003). Complete abrogation of infectivity was not achieved. In purple grape juice, 5% tartaric acid increased inactivation of *C. parvum* from around 57% to about 77%, whereas for white grape juice, addition of 5% tartaric acid increased inactivation of *C. parvum* viability from around 30% to about 55%. These data indicate that tartaric acid is probably unsuitable for treating FoNAO against FBP.

6. Thermal inactivation methodologies

6.1. Heat treatment

Adequate heat treatment can destroy parasites that contaminate FoNAO (Gajadhar, 2015). Table 2 presents available data on thermal treatments that have been reported for parasites on fresh produce. For all the high temperatures listed, it should be noted that these may be of minimal relevance for fresh produce such as salad vegetables and soft fruit that are intended for raw consumption.

Commercial pasteurization (71.7 °C, 15 s) has been demonstrated to be effective in inactivating *Cryptosporidium* oocysts in apple cider (Deng & Cliver, 2001), whereas blanching at 96 °C for 3 min has been shown to inactivate 93% of *Cryptosporidium* oocysts inoculated onto green peppers (Duhain et al., 2012).

Cyclospora cayetanensis oocysts appear to be susceptible only to higher temperatures. Heating *Cyclospora* oocysts on basil at 50 °C for 1 h did not prevent sporulation, but temperatures of 70 °C or 100 °C for 15 min, were effective in preventing sporulation (Sathyanarayanan & Ortega, 2006). For raspberries contaminated with *Eimeria acervulina* oocysts as *Cyclospora* surrogate, inactivation was achieved by heating to a minimal internal temperature of 80 °C maintained for 1 h (Lee & Lee, 2001).

Heat may not always be that effective at inactivating *Echinococcus multilocularis* eggs on FoNAO; although the eggs appear to lose infectivity at temperatures even as low as 43 °C, low humidity conditions may also be required (Veit et al., 1995). This aspect is important, particularly as *Echinococcus* eggs on fresh produce may be in contact with water droplets. Indeed, *E. multilocularis* eggs suspended in water have relatively high heat tolerance, showing infectivity after exposure to 65 °C for up to 120 min (Federer, Armua-Fernandez, Hoby, Wenker, & Deplazes, 2015). However, eggs were demonstrated to lose infectivity following heat exposure at 65 °C for 180 min or at 70, 75 and 80 °C for 30, 15 or 7.5 min (Federer et al., 2015). EFSA recommend heating of food for at least 3 h at 65 °C to ensure that potential contaminant *Echinococcus* eggs are inactivated (EFSA, 2018).

Microwave heating also has shown promise in the decontamination of some parasites on FoNAO, being effective in inactivating 93% of *Cryptosporidium* oocysts inoculated onto green peppers and microwaved in a domestic microwave oven (2450 MHz; 850 W) for 5 min. Pretreatment with chlorine increased the efficacy of inactivation to 98% (Duhain et al., 2012). These results suggest that industrial microwave blanching, as well as home microwave heating of fresh produce, could represent efficient treatments in parasite decontamination, should sufficient time and temperature variables be applied.

Few data are available on the effect of heat treatment on trematodes transmitted via FoNAO, such as *Fasciola hepatica* or *Fasciolopsis buski*. However, there are recommendations to boil water plants for 1–2 min prior to consumption to kill metacercaria of *F. buski* (Weng, Zhuang, Jiang, Lin, & Lin, 1989), or to cook aquatic plants thoroughly before eating, to control foodborne trematodes (Keiser & Utzinger, 2009).

6.2. Freezing

Freezing is another thermal methodology that can be efficient in controlling some parasites on FoNAO, although, as with heating, this treatment may not always be appropriate for FoNAO due to altered sensory properties (Gajadhar, 2015). Table 3 presents data on the control of parasites on fresh produce at varying combinations of time and low temperatures. *Cyclospora* oocysts on basil were able to sporulate following storage at -20 °C for up to 2 days, but were inactivated by exposure to -70 °C for 1 h (Sathyanarayanan & Ortega, 2006).

Oocysts of *C. parvum* and *T. gondii*, and *T. cruzi* trypomastigotes have also been shown to tolerate temperatures as low as -20 °C. For example, infectivity of *T. cruzi* trypomastigotes in açai pulp was unaffected by exposure to -20 °C for 26 h (Barbosa et al., 2012), and Duhain et al. (2012) reported that blast freezing at -20 °C for 4 min was not sufficient to kill *C. parvum* oocysts inoculated onto surfaces of green peppers (Duhain et al., 2012).

Regarding helminths, *Echinococcus multilocularis* eggs have been found to tolerate -18 °C for 240 days, but lost infectivity following exposure to -83 °C for 48 h (Veit et al., 1995).

As domestic freezers have average operating temperatures of between -13 °C and -18 °C (Evans, Foster, & Brown, 2014), household freezers should not be expected to inactivate all parasites in FoNAO. Indeed, EFSA recommend deep freezing at -80 °C for a minimum of 24 h in order to inactivate potentially contaminant *Echinococcus* eggs (EFSA, 2018); something that cannot be achieved in a domestic freezer. However, for industrial freezing, the paucity of information indicates that further studies are required.

7. Non-thermal inactivation methodologies

Table 4 presents results investigating the efficacy of various non-thermal technologies.

7.1. Drying

Among protozoan FBP, it has been shown that air-drying of *Giardia* cysts on lettuce results in viability abrogation within less than 24 h with almost 50% die-off of *Giardia* cysts recorded within the first 24 h; however, as the lettuce also became unpalatable under these conditions, it is not an appropriate methodology for this, and various other types of fresh produce (Utaaker, Skjerve, & Robertson, 2017). The effects of drying on other FBP on fresh produce are lacking, and it would be of particular interest to investigate how the viability of the more robust parasites (cestode and nematode eggs) are affected by the drying conditions used for producing “dried” fresh produce (e.g., dried fruit occurring in cereals, “trail mix”, herbs etc.). There have been various outbreaks of foodborne disease associated with dried fruits and vegetables, and although none of those reported are parasitoses, that various bacteria can survive drying processes indicates that this is also relevant

for parasite transmission stages (Bourdoux, Li, Rajkovic, Devlieghere, & Uyttendaele, 2016).

7.2. High-pressure processing (HPP)

In high-pressure processing (HPP), also known as pascalization, bridgmanization, or application of high hydrostatic pressure (HHP), foods are subjected to pressures from 100 to 800 MPa. The processing temperature during treatment can be from below 0 °C to above 100 °C, with exposure times ranging from a few seconds to 20 min and longer, depending on process conditions. Nutritive values and sensory properties of food often remain unaffected by HPP.

The studies on the effectiveness of HPP in inactivating FBP have often used surrogate organisms, particularly for helminths, and often have not investigated the process on a relevant FoNAO matrix, although initial data have suggested that this technology could be of use for inactivating nematode eggs contaminating FoNAO (Rosypal, Bowman, Holliman, Flick, & Lindsay, 2007).

With regards to protozoa, Slifko et al. (2000) evaluated the effects of HHP at 550 MPa for 0, 30, 45, 60, 90, and 120 s on *C. parvum* oocysts suspended in apple and orange juice. HHP inactivated *C. parvum* oocysts by at least 3.4 log after 30 s of treatment, and after at least 60 s of treatment more than 4.2 log inactivation was observed as assessed by cell culture (Slifko, Raghubeer, & Rose, 2000).

For *Toxoplasma gondii* oocysts, Lindsay et al. (2008) inoculated raspberries with 5×10^4 oocysts of the *T. gondii* VEG strain exposed to 500, 400, 340, 300, 270, 250, 200, 100 MPa for 60 s and showed 340 MPa resulted in the oocysts becoming non-infectious for mice (Lindsay et al., 2008).

For *Cyclospora*, the only investigations on HPP have used *Eimeria acervulina* oocysts as a surrogate. Sporulated *E. acervulina* oocysts inoculated onto basil and raspberries at high (10^6 oocysts) and low (10^4 oocysts) levels and treated with 550 MPa at 40 °C for 2 min resulted in apparent abolition of infectivity to chickens (Kniel, Shearer, Cascarino, Wilkins, & Jenkins, 2007).

7.3. X-ray irradiation

X-rays are produced by reflecting a high-energy stream of electrons off a target substance (usually one of the heavy metals) into foods. The adverse effects on nutritional or organoleptic (sensory) properties associated with thermal processes are likely to be reduced as x-rays do not produce heat.

Although some studies on inactivation of FBP by x-ray treatment have been performed, none have been conducted on parasites contaminating FoNAO with the intention of investigating the suitability of this process for food treatment.

7.4. Gamma irradiation

Gamma rays used for food treatments are emitted from radioactive forms of the radionuclide elements, usually cobalt-60 or, very rarely, caesium-137. A few studies have investigated the effects of gamma irradiation on survival of FBP as contaminants of fresh produce, including *Toxoplasma* oocysts on raspberries irradiated with doses of ≥ 0.4 kGy (cesium-137) failed to cause infections in mice (Dubey, Thayer, Speer, & Shen, 1998), despite antibodies being produced in some mice. Lacombe et al. (2017) showed that irradiation at ≥ 0.2 kGy (caesium-137) led to at least 4 log PFU/g inactivation of *Toxoplasma* oocysts on blueberries as evaluated by cell culture plaque assay (Lacombe et al., 2017). For *Cyclospora* oocysts, the only data rely on experiments using *Eimeria* oocysts as surrogates, inoculated onto raspberries and these indicate that irradiation at 1 kGy or higher is needed to ensure complete abrogation of infectivity (Lee & Lee, 2001).

7.5. Ultraviolet irradiation

The effect of ultraviolet irradiation (UV) on the viability and infectivity of FBP is diverse and related to parasite species, stage, and the matrix assayed. Reductions in infectivity have been observed for protozoa that may be transmitted by FoNAO, but inactivation varies depending on the species tested and the matrix; the sensitivity of *Cryptosporidium* and *Giardia* to UV light has resulted in this becoming a standard treatment for drinking water. However, extrapolation to parasites as contaminants of food is less clear, particularly due to shading effects. However, experiments with raspberries spiked with *Cryptosporidium* oocysts and exposed to a pulsed UV light of total fluence $4\text{J}/\text{cm}^2$, have reported a 2 log reduction or 3 log reduction in infectivity, with initial levels of 10^3 or 10^4 oocysts respectively (Le Goff et al., 2015). Raspberries have also been spiked with 2×10^4 oocysts *Eimeria acervulina* oocysts, as a surrogate organism for *Cyclospora*, and treated with UV at doses up to $261\text{mw}/\text{cm}^2$; in this experiment, infectivity was reduced at the higher UV doses, as demonstrated by lower oocyst excretion in infected chickens and a lower lesion score at autopsy; however when the inoculum was 2×10^6 oocysts, oocyst excretion was not affected (Kniel et al., 2007). In experiments with a similar set up, but 2×10^6 oocysts inoculated onto basil leaves, similar results were obtained as for raspberries, but when the inoculum size was lower (2×10^4 oocysts), a UV dose of $160\text{mw}/\text{cm}^2$ was used, the effect was greater on the oocysts on the basil than on the raspberries (Kniel et al., 2007). As discussed by the authors, this may well be due to the shadowing effect and that basil provides a planar surface for irradiation, where as raspberries are irregular and oocysts in crevices between drupules may avoid the UV irradiation.

Helminths seem to be less sensitive to UV exposure than protozoa, although data from food matrices is lacking. *Ascaris* eggs are among the most UV-resistant pathogens, although exposure to $400\text{mJ}/\text{cm}^2$ can reduce embryonation of *Ascaris suum* by at least 2.23 logs (Brownell & Nelson, 2006).

7.6. Ultrasound

Ultrasound is used in many applications in the food industry. Depending on the frequency used, ultrasound may be used to stimulate cellular activity, for extraction and emulsification, and for cellular destruction and cleaning, among others (Mason, Paniwnyk, & Lorimer, 1996). Ultrasound parasite inactivation tests on fresh produce are lacking, but experiments conducted on aqueous matrices have suggested that ultrasound may affect the viability of *Cryptosporidium* oocysts. Continuous sonication of real and filtered municipal water treatment plants effluents with 20 kHz power for 10 min was able to achieve around 1 log reduction of viability of *C. parvum* oocysts (Abeledo-Lameiro, Ares-Mazás, & Gómez-Couso, 2018).

7.7. Supercritical fluid-state gases

Supercritical fluids exist when the temperature and pressure are such that distinct liquid and gas phases do not exist; the temperature (and thus molecular kinetic energy) is sufficiently high that condensation will not occur, but the pressure is sufficiently high that the substance does not remain in pure gaseous state. Although supercritical fluid-state gases have properties suitable for various applications, the mechanisms behind their known antimicrobial effects are poorly understood. Supercritical carbon dioxide (SC CO_2) treatment has been recognized as a promising non-thermal treatment technology for the food industry, particularly for extraction process, but also inactivating a large variety of microorganisms, particularly in combination with ultrasound (Koubaa, Mhemdi, & Fages, 2018). More recently, an alternative supercritical fluid, supercritical nitrous oxide (SC N_2O), has been shown to have a similar microbicidal effect as that of SC CO_2 , but without affecting pH change. Although we are lacking information on

use of this technology on parasites in food matrices, the efficacy of supercritical CO_2 and N_2O at inactivating *Ascaris suum* eggs has been investigated, and a strong oocidal effect was observed after 1 min exposure, with both gases achieving 2.4 log inactivation evaluated by embryonation (Mun, Lee, & Yoon, 2012).

8. Future trends and novel technologies in inactivation of parasites on food

Better understanding of the extent of contamination of FoNAO and survival of transmission stages on these matrices, along with methods for inactivation of potentially contaminated produce and assessment of the efficacy of such methods, are important for safeguarding public health. However, the application of Good Agricultural practices and HACCP approaches are essential for minimizing contamination of FoNAO.

Due to the lack of efficacy of chlorine-based traditional processes for disinfection of FBP in the fresh produce industries, coupled with the need to find alternatives to chlorine for health concerns, other directions have been explored (De Corato, 2019). Temperature (heat, freezing) and desiccation may be appropriate for some FoNAO, but not appropriate for produce such as leafy salad vegetables or fresh berries. Although technologies such as irradiation technologies (X-ray, gamma, UV), high pressure processing, and ultrasound may be effective, these are also associated with some difficulties, related to their efficacy on food matrices, their applicability within an industrial food setting, and their effects on the organoleptic and sensory properties of the FoNAO. Although X-ray is reliable and efficient at processing large volumes of produce, there is considerable consumer mistrust and its use is relatively expensive. Use of electron beam as alternative irradiation source to gamma has not yet been investigated for inactivation of FBP on FoNAO but some characteristics of this technology indicate that it may provide more rapid effects. Various novel technologies have been investigated for other pathogens, such as bacteria, that may be transmitted via FoNAO but have yet to be investigated for parasites. Such technologies include non-thermal/cold plasma and plasma activated water. For both of these, reactive oxygen species (ROS) are usually considered as the major inactivating agent in combination with a high positive oxidation reduction potential and low pH resulting in oxidative stress in microbial cells, and thus damage to DNA, proteins, and lipids (Ma et al., 2015). Another technology that may be of relevance is pulsed electric field (PEF) technology; whereas PEF has been considered as an important, non-thermal food processing technology for decades, particularly for beverage production (Aneja, Dhiman, Aggarwal, & Aneja, 2014); advances in this methodology, such as nanosecond PEF (nsPEF) and microchip PEF (MCPEF) are considered to be effective membrane disruptors. These may have better potential for eliminating microbial contaminants, either alone or in combination with other technologies, without affecting sensory qualities (Zhu et al., 2019).

Although, data on the use of these novel methods against other foodborne pathogens is being steadily accrued, for FBP the data tend to be scattered, inconsistent, or, more often, entirely absent. One important aspect is that suitable methods for determining survival and infectivity of relevant FBP will need to be developed, such that we have a good basis for evaluating the effects of such exciting technologies on this important pathogen group in FoNAO (Rousseau et al., 2018). Without appropriate viability/infectivity assessment methods, it is very difficult to determine the efficacy of novel methods; for most parasites, infectivity remains the gold standard – but this has associated ethical issues, and is not possible for parasites such as *Cyclospora*, for which no animal model is available. In parallel, investigations on the use of appropriate surrogates, may help to circumvent the difficulty in obtaining some parasites and would help in assessing the efficacy of processes at industrial scale for relevant parasitic targets (Busta et al., 2003).

9. Conclusions

Based on our extensive literature review, information on the effects of different inactivation techniques on relevant parasites on FoNAO has been assimilated. Given that contamination of FoNAO with transmission stages of FBP occurs relatively frequently, that they survive well under storage, and are difficult to remove by washing procedures, it is important to consider the efficacy of potential inactivation methods that may be used on FoNAO without affecting their intrinsic qualities.

Chemical oxidizers have some effects on survival of FBP, but the concentrations and contact times required to ensure inactivation are not appropriate for FoNAO. Organic acids and other chemicals are ineffective at inactivating FBP on FoNAO. Data from water studies suggest that ozone may be suitable for inactivation of several foodborne parasites, and could be promising for use in fresh produce industries. The application conditions (around 5 °C) and the requirement to maintain the aqueous ozone concentration among various levels of organic matter are critical parameters in the context of minimally processed fresh produce industries. Citric acid studies have investigated the effects of various parameters, but none demonstrated total elimination of *C. parvum* oocyst viability, indicating that treatment of FoNAO with citric acid would be unlikely to reduce the risk of infection should the food be contaminated with viable oocysts.

Among physical treatments, heat particularly, and for some parasites, freezing and desiccation may be effective, but are not appropriate for FoNAO that are to be eaten raw, such as lettuce or berries. And some important FBP, such as *Echinococcus* eggs, are resistant to elevated or decreased temperatures. Although technologies such as irradiation technologies (X-ray, gamma, UV), high pressure processing, and ultrasound may be effective, the data are sparse and mostly derived from water matrices rather than actual FoNAO, and the range of FBP tested are limited. Furthermore, these technologies are associated with some difficulties, associated with applicability within an industrial food setting, cost and effect on the organoleptic and sensory properties of the FoNAO, but also consumer perception limiting the applicability of irradiation technologies.

This assimilation of data indicates that research on the inactivation of FBP transmitted by FoNAO should be expanded, with efforts directed towards developing reliable and useful methods for assessing effects of methods, such that investigations of current and novel inactivation methods used for other pathogens on their effects on parasites in FoNAO enables the production of useful, comparable data of relevance for industrial applications.

Declaration of interests

The authors declare that they have no conflict of interests.

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